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TABLE OF CONTENTS

VOLUME 208, 1980

Review article Plasmapheresis—Bloodletting revived and refined	1
I M Nilsson and S Lamme On acquired hemophilia A. A survey of 11 cases	5
L Brandt and P G Nilsson Lymphocytopenia preceding chronic lymphocytic leukemia	13
S Ljungman M Aurell M Hartford J Wikstrand L Wilhelmson and G Berglund Blood pressure and renal function	17
R Larsson G Bodemar B Kagedal and A Wulan The effects of cimetidine (Tagamet®) on renal function in patients with renal failure	27
R Hultgren L Lundin L E Roxin and P Venge Serum and urinary myoglobin in alcoholics	33
S Kaulola V Manninen and P I Halonen Serum lipids with special reference to HDL cholesterol and triglycerides in young male survivors of acute myocardial infarction	41
J Tuomilehto E Voutilainen J Huttunen S Vinni and J Homan Effect of ginseng on body weight and serum lipids in hypercholesterolemic females	45
A Wu J A Lee A R Aroeggaard and B Holst Hydralazine in arterial hypertension. Randomized double-blind comparison of conventional slow-release formulation and of bid liquid dosage regimens	49
S Akre C Helmers and J Lundman QT intervals at discharge after acute myocardial infarction and long-term prognosis	55
J C Mogelvang E A Petersen P E Folke and L Olesen Antiarrhythmic properties of a neuroleptic butyrophenone, melperone, in acute myocardial infarction. A double-blind trial	61
M Waller and P Thorsen Ventricular arrhythmias during exercise testing and 24-hour ECG tape recording in patients with valvular heart disease and in normal individuals	65
B E Nilsson and J J Abdom Epitaxial cardiac arrhythmias and accident rate	69
A J Al-Lam and B E Nilsson Epitaxial cardiac arrhythmias and femoral neck fracture	73
B O Olsson P Andersson and E Swartz Autonomic regulation and myocardial conduction in lateral wall infarction with preserved ejection	—
M von Arden M Britton L de Figue C Helmers V Mah and S Murray A study of a risk patients treated in a coronary care unit and in general medical wards	81
G Toss S Aronson L Larsson and H Larsson Serum D-phenylalanine in women with epilepsy for the aged	87
J-G Lapeere and G Koster Serum thyroxine, triiodothyronine and thyrotropin releasing hormone in hyperthyroidism	94
M Gennari L Paganini L Gennari J M Landberg T Hultén A Rönner S Pahl G Landberg J E Jönsson P Jönsson and E Gennari Report on a patient with primary carbonic anhydrase deficiency, apocatalytic and apocatalytic carbonic anhydrase deficiency	97
L Ahlström S Eklund P O Carlsson L Lundin P E Liss and H Liss Postoperative localization of diaphragm and phrenic nerve and phrenic nerve sensitivity of diaphragm during acute or chronic pain in the chest wall and phrenic nerve	—

<i>P Stavem J Frøyskov Larsen B Lø and T O Rørvik</i> Amyloid deposits in bone marrow aspirates in primary amyloidosis	111
<i>P Stavem B Lø and T O Rørvik</i> Abnormal pattern of the rough endoplasmic reticulum of plasma cells in multiple myeloma with multiple concentric lamellar bodies and single sac loops	115
<i>H Kræmmer Nielsen</i> Multifocal idiopathic fibrosclerosis Two cases with simultaneous occurrence of retroperitoneal fibrosis and Riedel's thyroiditis	119
<i>H Draminsky Petersen and M Bergman</i> Cortisone induced remission of hypothyroidism in Schmidt's syndrome	125
<i>G Nilsson</i> Symptomatic diabetes mellitus cured by potassium and withdrawal of polythiazide in a hypokalemic hypertensive woman	129
<i>J Murros and A Luomanmaki</i> A case of hypocalcemia heart failure and exceptional repolarization disturbances	133
<i>J B Reitan E Pape S D Fossa O J Julsrud O A Slettnes and O P Solheim</i> Osteosclerotic myeloma with polyneuropathy	137
<i>B A Afzelius L Enet J Palmblad A M Uden and N Venizelos</i> Structure and function of neutrophil leukocytes from patients with the immotile-cilia syndrome	145
<i>S Ingimarsson A Bergström L Å Broström A Cantell and H Strander</i> Effect of long term treatment with human leukocyte interferon on various laboratory parameters	155
<i>B Christensson G Matell and P Biberfeld</i> Immunological studies on human thymus Occurrence and distribution of immunoglobulins and immunological receptors in myasthenia gravis and control patients	161
<i>J Weits G C de Gast T H The M van der Giessen T Ockhuijsen J J M Festen and E Mandema</i> High immune responsiveness in a family with multiple paraproteinaemia and autoimmune thyroid disease	169
<i>L E Wille E Olsen O Førre A Sletten and H Jentoft</i> Demonstration and partial characterization of an atypical protein in the urine of a patient with primary amyloidosis	177
<i>E F Mogensen and A Green</i> The epidemiology of thyrotoxicosis in Denmark Incidence and geographical variation in the Funen region 1972-1974	183
<i>G Holm S Johansson A Vedin C Wilhelmsson and U Smith</i> The effect of beta-blockade on glucose tolerance and insulin release in adult diabetes	187
<i>B von Bonsdorff and R Gordin</i> Castle's test (with vitamin B ₁₂ and normal gastric juice) in the ileum in patients with genuine and patients with tapeworm pernicious anaemia	193
<i>J Savar S Skrede J Eriksen and J P Blomhoff</i> The relation between the levels of HDL cholesterol and the capacity for removal of triglycerides	199
<i>J Persson and G Fax</i> HDL-increasing effect of cyclofenil	205
<i>P O Wester</i> Urinary zinc excretion during treatment with different diuretics	209
<i>M Hellerstedt R Jonasson and E Orinius</i> Electrocardiographic diagnosis of ventricular septal infarction	213
<i>D Bone P Carlens A Holmgren H Johansson R Jonasson J Kandilas L Mogensen R Nordlander and E Orinius</i> Thallium 201 scintigraphy after acute myocardial infarction	219
<i>S Ahne L Erhardt T Lundman N Rehnqvist and A Sjögren</i> Effect of metoprolol on QT intervals after acute myocardial infarction	223
<i>J Schnohr P Grande and C Christiansen</i> Enzyme activities in serum after extensive exercise with special reference to creatine kinase MB	229
<i>G Aasen and H M M Frey</i> Excessive sensitivity to the hyponatremic effect of chlorpropamide in a patient with diabetes mellitus and anterior pituitary insufficiency	233
<i>B Persson A Rausing I Tureson and O Zettervall</i> Predominant B lymphocyte deficiency in a case with lymph node disease resembling angioimmunoblastic lymphadenopathy	237
<i>Editorial</i> To treat or not to treat—is this the question?	241
<i>M Schroll</i> Smoking habits in the Glostrup population of men and women born in 1914 Implications for health evaluated from ten year mortality incidence of cardiovascular manifestations and pulmonary function 1964-1974	245
<i>J W Paulsen</i> Coronary ischaemia and occlusion in giant cell (temporal) arteritis	257
<i>P O Wester</i> Zinc balance before and during treatment with bendroflumethiazide	265
<i>P O Wester</i> Tissue zinc at autopsy—Relation to medication with diuretics	269

<i>N C Henningsen B Bergengren O Malmborg O Pihl A Renmarker and L Strand</i>	Effects of mefruside treatment in hypertension	273
<i>P Ylitalo A Pasternack S Kallio T Vantunen and T Metsa Ketela</i>	Increased urinary protein excretion after intravenous injection of furosemide in man	279
<i>J Hiras M Enckell B Kuhlback and A Pasternack</i>	Psychological and social problems encountered in active treatment of chronic uraemia III Prediction of the living donor's psychological reaction	285
<i>E Lindstrom and F D Lindstrom</i>	Skeletal scintigraphy with technetium diphosphonate in multiple myeloma—a comparison with skeletal X ray	289
<i>L Baldetorp and J Martensson</i>	Urinary excretion of inorganic sulfate ester sulfate total sulfur and taurine in cancer patients	293
<i>P Arner P Engfeldt and J Ostman</i>	Blood glucose control and lipolysis in diabetes mellitus	297
<i>G Sartor A Melander B Schersten and E Wahlén Boll</i>	Comparative single dose kinetics and effects of four sulfonylureas in healthy volunteers	301
<i>G Blohme K Karlsson and J Waldenström</i>	Early insulin response in latent gestational diabetes	309
<i>F Lithner and N Tornblom</i>	Gangrene localized to the lower limbs in diabetics	315
<i>H Hev and L Tougaard</i>	Elevated bone phosphorus/hydroxyproline ratio following jejunioileal bypass surgery	321
<i>S J Hoorntje A J M Donker E J L Prins and J J Weening</i>	Membranous glomerulopathy in a patient on captopril	325
<i>J Asplund</i>	Pseudotumor cerebri in pseudohypoparathyroidism	331
<i>H G Levander</i>	Granulomatous hepatitis in a patient receiving carbamazepine	333
<i>✓ D V Hamilton E J A Lea and S P Jones</i>	Dietary fatty acids and ischaemic heart disease	337
<i>✓ G Thompson</i>	The lipid hypothesis	341
<i>E Simonsen J Strøde Nielsen and B Lyager Nielsen</i>	Sinus node dysfunction in 128 patients A retrospective study with follow up	343
<i>D Schlossman K Selin J Wallin and I Wallentin</i>	The diagnosis of atrial myxomas Difficulties and pitfalls	349
<i>L Eriksson and O Pahlm</i>	The clinical impact of long term ECG recording A retrospective study of 150 patients	355
<i>A Terent and B Andersson</i>	The outcome of patients with transient ischemic attacks and stroke treated with anticoagulants	359
<i>T Thulin M Abdulla I Dencker M Jägerstad A Melander Å Norden B Schersten and B Åkesson</i>	Comparison of energy and nutrient intakes in women with high and low blood pressure levels	367
<i>D E H Andersson and S Rojdmarm</i>	Effect of verapamil on blood glucose and serum insulin in patients with hyper and hypothyroidism	375
<i>G Bucht A Wahlén T Wentzell and B Winblad</i>	Renal function and morphology in long term lithium and combined lithium neuroleptic treatment	381
<i>R Adolfsson G Bucht E Lithner and B Winblad</i>	Hypoglycemia in Alzheimer's disease Preliminary report	387
<i>✓ Vaaler K F Hanssen and Ø Aagenæs</i>	Effect of different kinds of fibre on postprandial blood glucose in insulin-dependent diabetics	389
<i>R Norberg F A Wollheim and P O Gedda</i>	Circulating protein complexes in D-penicillamine therapy of rheumatoid arthritis Correlation between IgG and α_1 antitrypsin IgA complexes and clinical response	393
<i>N Stjernberg H Bjørnstad Pettersen and H Truedsson</i>	Flexible fiberoptic bronchoscopy in sarcoidosis	397
<i>N Kromann and A Green</i>	Epidemiological studies in the Upernavik district Greenland Incidence of some chronic diseases 1950–1974	401
<i>B G Persson M Donner B Petersson B Eklof and A Wentzell</i>	Aneurysm of the popliteal vein as a cause of pulmonary embolism	407
<i>M Peters and I R Mackay</i>	Multiple myeloma and gastric carcinoma Possible late effects of limited abdominal X irradiation	411
<i>C Bengtsson U Bengtsson and K Lincoln</i>	Bacteriuria in a population sample of women	

Prevalence characteristics results of treatment and prognosis	417
<i>U Dahlström J Martensson and F D Lindström</i> Occurrence of adult Fanconi syndrome in benign monoclonal gammopathy	425
<i>M Miettinen L Saxen and E Saxen</i> Lymph node toxoplasmosis Follow up of 237 histologically diagnosed and serologically verified cases	431
<i>F A Rømer</i> Angiotensin-converting enzyme in newly detected sarcoidosis With special reference to enzyme levels in patients with erythema nodosum	437
<i>G Evinsson and E Ornnus</i> Prodromal ventricular premature beats preceded by a diastolic wave	445
<i>T Deckert J Boysen J Sandahl Christiansen A Kølendorf P Aaby Svendsen and A R Andersen</i> 24-Hour blood glucose profiles in insulin-dependent diabetics treated with intravenous insulin infusion systems A comparison between closed and open loop systems	451
<i>J Lidbeck</i> Studies on hemopoietic dysplasia (the preleukemic syndrome) Clinical course and prognostic factors in 42 patients with dysplastic bone marrow	459
<i>E Simonsen B Løager Nielsen and J Stræde Nielsen</i> Sinus node dysfunction in acute myocardial infarction	463
<i>H Åberg H Hedstrand and H Lithell</i> Blood pressure control in a middle aged male population A 6-9-year follow up with special reference to the problem of non responders	467
<i>Danish Multicenter Study</i> Emergency treatment of severe hypertension evaluated in a randomized study Effect of rest and furosemide and a randomized evaluation of chlorpromazine dihydralazine and diazoxide	473
<i>S Julius L Hansson L Andren T Guldbrandsson R Sierstson and A Siennson</i> Border line hypertension Hypertension seminars at Östra Hospital Göteborg Sweden	481
<i>L Tarssanen M Huikkio and M Rossi</i> Amiloride induced hyponatremia	491
<i>A Ponkū T Pitkanen T Pettersson S Aittoniemi and T U Kosunen</i> Carditis and arthritis associated with <i>Campylobacter jejuni</i> infection	495

REVIEW ARTICLE

Plasmapheresis—Bloodletting Revived and Refined

During several centuries one of the chief therapeutic methods besides the administration of herbs was attempts to remove noxious substances from the body. This was performed either by purgation often with drastic laxatives or still more dramatic by bloodletting. It is said that several important persons, for instance the French king Louis XIII, were probably killed by such therapy. Fifty years ago we were still taught that bloodletting might be valuable in certain states of cardiac decompensation but the advent of rapidly acting diuretics has made even this last remnant of an old phlebotomy obsolete. In recent years however removal of plasma by so-called plasmapheresis has become highly fashionable. The risks of an overzealous application are as great or even greater than they were regarding phlebotomy but there is no question that selected patients may be much improved by such treatment. It may even be life saving.

Initially I should like to stress that the word plasmapheresis consists of two halves: plasma and apheresis, a Greek word that means removal. The etymological hyphenation should therefore be plasm-aph-eresis. The correct spelling is also leukapheresis not leukophoresis when white cells are removed and utilized.

As early as 1947 Lerner and Watson (3) described the occurrence of what they called cryoglobulin, i.e. globulin that precipitates on cooling. Such precipitation had been described before as stratification of serum or complete gelification (8). This author determined serum viscosity at different temperatures and found sera with very rapid increase at low temperatures but without the formation of precipitates. Together with Willert we tried plasmapheresis on a patient with macroglobulinemia in 1955 and were able to show that the macroglobulin content could be diminished by our technique was not radical enough. John Fahey and his group in Bethesda performed very massive plasmapheresis on some patients with highly viscous serum containing large amounts of macro-

globulins with excellent clinical results (7). At this time the technique with plastic bags was used and the separation of plasma from the red cells was a complicated and very time-consuming business.

The advent of the cell separator changed this completely and it became easy to withdraw large quantities of plasma separated from the cells. The indications for removal of plasma and the following substitution with normal plasma or human serum albumin have increased considerably during the last years. It has been found that the high viscosity may be detrimental in several ways. Strangely enough the high viscosity in itself that should cause mechanical hindrance of the circulation with an increased load upon the heart is rare even if convective results after plasmapheresis have been described. It seems to be a paradox that the blood is diluted in hyperviscous states but this is a fact. Many of these patients suffer from considerable "anemia" and have even been treated with blood transfusions when the opposite method removal of plasma should have improved the so-called anemia. The volume of total erythrocytes may be practically normal whereas the plasma volume may be doubled. We have been able to observe this and have seen a cure of anemia after removal of 10 liters of plasma. The third and perhaps most important symptom in the hyperviscosity syndrome is the bleeding tendency. Already in the first publication on macroglobulinemia we described nosebleeds, oozing of blood from the gums and intraocular bleeding. As a matter of fact this is one of the most important clinical symptoms. It occurs also in a few patients with a very high content of viscous IgG produced by myeloma cells. The picture in the eye grounds and the bleeding from the mucous membranes are therefore general signs of hyperviscosity and it should be remembered that one of the most important diagnostic procedures is ophthalmoscopy. The recommendations of what I have called fundus viscoproteinicus are of great practical value.

Regarding indications for removal of plasma it

may be said that this should only be used in severe conditions with marked hyperviscosity when rapid improvement is necessary. The method is not a treatment of the underlying disease and it is clear that this should be attacked by cytostatic drugs and possibly also by glucocorticoids. Before the advent of active plasmapheresis on a grand scale we have seen very marked reduction of a high plasma viscosity during treatment with chlorambucil or melphalan with lasting results regarding the viscosity syndrome even if it takes a long time to obtain such results.

Another type of cryoglobulinemia has recently been the subject of intense study. These patients have smaller amounts of cryoglobulin and this is formed by immune complexes. These usually contain an active IgM that is regarded as an antibody against IgG. In the classical case the IgM is monotypic and is probably produced by one clone of cells. The condition is then an instance of macroglobulinemia with the formation of an immunologically active IgM. This may well be compared to rheumatoid factor activity and as a matter of fact it is a characteristic of such sera that they have a high titer of so called rheumatoid factor. The clinical picture in this syndrome is characterized by purpura, arthralgia and often involvement of the kidneys. Sometimes a picture of polyarteritis nodosa is also seen and the first authors to study the occurrence of cryoglobulins in different clinical conditions found some patients with this disease (4).

The story of these immune complexes has recently become still more complex when it was found that a large number of these patients had liver disease with evidence of infection with hepatitis B virus. A recent study from Franklin's Laboratory at the NYU showed that 74% of the cryo-precipitates contained either the antigen or its antibody. Four precipitates were examined by electron microscopy and virus particles were found in all. It is well known that there is a connexion between hepatitis B virus and polyarteritis nodosa but it is quite obscure why the immune response to the virus may result in IgM anti IgG giving characteristic cryoglobulins. To my mind this is one of the most fascinating developments in recent studies of infectious diseases: the damage may be caused not so much by the infectious agents as by the response of the body to the infection. New such examples are being discovered every year (4).

It is clear that all immune complexes are not cold precipitable. Some years ago we treated two patients who were sent to us because they had a severe hyperviscosity syndrome with anaemia spuria and a markedly increased plasma volume. One of these patients was treated with plasmapheresis and was much improved. The sera contained immune complexes of different sizes. It is possible that these conditions are not readily recognized by the clinicians and we have only seen these two patients with the condition in amplissima forma. Their histories have been published (6).

The most fascinating application of bloodletting with the new techniques regards another type of disease. The number of patients who are suffering from deficiencies because of antibodies against metabolically important substances is ever increasing. A special group is formed by patients who suffer from severe bleeding tendency because they have developed antibodies against some coagulation factor. These conditions are extremely difficult to treat because the antibody neutralizes exogenous clotting factors administered intravenously. Usually these antibodies arise spontaneously. They are true autoantibodies but the facts regarding their possible monoclonality have not yet been settled. The few instances where this has been definitely demonstrated would be compatible with the hypothesis that fortuitously a special clone of immunoglobulin producing cells proliferates. They would thus belong to the so called monoclonal gammopathies. In some other conditions the patients suffer from classical autoimmune disease such as SLE and then the immunoglobulin production is polyclonal.

Sick receptors on cell surfaces is an interesting subject that has lately become very fashionable. J. Roth and Quatecasas were the first authors who realized that marked refractoriness to insulin may be caused not only by circulating antibodies against the hormone. Such antibodies can be detected *in vitro* by their activity in neutralizing the effects of insulin. It has been demonstrated beyond doubt that there are also patients who are resistant against very large doses of insulin and still have no freely circulating antibodies against the hormone. It seems pretty clear that antibodies may bind to the insulin receptors on the cells and thus cause a block. I feel convinced that this concept of receptor blocking by specific antibodies will become one of the most fruitful fields for extended studies in the future.

Another similar situation is probably related to the development of myasthenia gravis. Many authors have maintained that this disease has an immunological background and it is well known that the thymus is in some cases implied in the pathogenesis. Antibodies against the acetylcholine receptor on the neuromuscular junction are probably responsible for the disease in a majority of cases.

The best proof that this theory is correct may of course be obtained if massive plasmapheresis would be successful. A number of such patients have now been observed and as usually the enthusiasm was very great initially whereas the present opinion is more balanced (2). Even if the practical value of the method may be discussed there is no question about the fact that plasmapheresis strongly influences the severity of the disease and the next years will teach us the correct selection of cases and the best technique. Theoretically attractive is the hypothesis that massive plasmapheresis should be followed by a course of cytostatics for instance azathioprine in order to damage the clone(s) that produces the antibody. It may perhaps be specially vulnerable after decided lowering of its product i.e. the noxious IgG and cytostatic treatment should therefore be given directly after plasmapheresis. Similar ideas may of course be applied to the rare cases with refractoriness against insulin when this is caused by an autoantibody against the receptor.

The discussion about cytotoxic antibodies in autoimmune disease has been very lively during the last decades especially regarding SLE. Attempts to influence the symptoms in this disease by plasmapheresis have been made but do not seem to have been successful. There is however another condition where the effects from removal of an autoantibody seem to have been excellent.

Goodpasture's syndrome is a rare condition with chronic purpura of the lungs (so-called hemosiderosis pulmonum) with repeated hemoptyses combined with a renal disease that is often fatal. In some instances the pulmonary damage occurs alone and may be diagnosed clinically (9). It is also in itself a serious disease. Immunological studies have detected the presence of an autoantibody against basement membrane both in kidney and lungs. There is obviously a chemical similarity or identity between some constituents of this membrane in both organs. Several reports seem to indi-

cate that massive plasmaphereses really improve the situation. Combined with cytostatics it may even cause remissions or possibly cures (5).

It is interesting to realize that complete Goodpasture's syndrome is not always present. In 1944 the present author described the clinical diagnosis of a patient with the typical picture of hemosiderosis pulmonum who had been seen in Uppsala (8). In 1948 the literature on pulmonary hemosiderosis was collected and at that time nothing was known about the combination with renal disease. Later I have seen in various countries examples of pure hemosiderosis pulmonum and it is quite evident that a number of patients who should be treated and possibly saved by plasmapheresis will occur in departments of medicine and pneumonology as obscure hemoptysis with widespread pulmonary infiltrates. It will be interesting to see when the first patient with purely pulmonary symptoms will be treated successfully. Also the pediatricians have published observations of this disease.

Even if there can be no question about the fact that plasmapheresis as a new form of bloodletting has come to stay as an important therapeutic procedure we must remember that the indications should be very clear before we start. The method is not without risks if complications are not observed and corrected at an early stage. The method is extremely expensive both as regards plasma substitutes (fresh plasma serum albumin) and above all regarding manpower. The method should only be used on very strong indications. It is not a cure of macroglobulinemia or myeloma but may have a place in alleviating severe symptoms in two stages of these diseases. 1) The acute stage if severe hyperviscosity is present and needs quick relief until cytostatics have given results. 2) The final stage when hyperviscosity recurs and the bone marrow has been so severely damaged either by the disease or by previous therapy that this can no longer be continued.

Jan G Waldenström

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BOOK REVIEW

Bradykinin, Kallidin and Kallikrein. Handbook of Experimental Pharmacology, vol XXV, Supplement Edited by E G Erdos 656 pages with indexes 817 Sw cr 1274 Springer Verlag Berlin Heidelberg and New York 1979

The same subject was covered in vol XXV in the Handbook of Experimental Pharmacology series published in 1970. During the last decade research within this field has been quite rapid and an updating edition is highly adequate. The new volume is most impressive both from qualitative and quantitative point of view. The book is organized in 17 chapters written by well known investigators in the respective fields. They deal with various aspects from basic and clinical point of view.

The first chapters are mainly concerned with the biochemistry of kininogenases, i.e. the enzymes releasing kinins and their inhibitors, and also discuss closely related systems. These parts are of special value for investigators actively engaged in research but serve also as a basis for the physiological and clinical aspects presented in the following sections. Of particular clinical interest and relevance are chapters dealing with plasma kallikreins, kininases, renal kallikreins and bradykinin-prostaglandin interactions.

The interrelations between the plasma kallikrein-kinin system, blood clotting, the complement system and the fibrinolytic system have to a great extent been clarified during the last few years. This is of importance with respect for instance to isolation and purification of plasma components to be administered to patients with various deficiencies.

Kinin-degrading enzymes, kininases, attract consider-

able interest because of the recent finding that kininase II is identical with the angiotensin converting enzyme. Inhibitors of kininase II decrease blood pressure in renovascular hypertension and also in many cases of essential hypertension, particularly those with elevated plasma renin activity. This has led to a new intriguing approach in antihypertensive therapy.

The renal kallikrein-kinin system has been extensively studied during the last decade. 30 years after the original description of the urinary kallikrein. The role of this system in renal handling of electrolytes and water and in blood pressure regulation is thoroughly penetrated by Ward and Margolius and also by Mills.

The finding by Vane in 1971 that aspirin-like drugs inhibit prostaglandin synthesis has led to intense research within this field and it has become clear that many effects of bradykinin are mediated by prostaglandins. The current knowledge in this area has been summarized by Terragno.

In general the various chapters are well written and clear both with respect to text, tables and illustrations. In most instances the authors have included an impressive number of references. Actually the author index contains more than 6000 references altogether and the subject index covers 76 pages.

In conclusion this volume is of major general interest and value as a handbook for investigators in the field. However in view of the increasing importance of bradykinin, kallidin and kallikrein for the understanding of various pathological states, it can also be recommended for doctors working in various areas of clinical medicine.

Lennart Hulthén and Bernd Holmfelt, Malmö, Sweden

On Acquired Hemophilia A

A Survey of 11 Cases

Inga Marie Nilsson and Stefan Lammé

From the Coagulation Laboratory, Malmö General Hospital, Malmö, Sweden

ABSTRACT Acquired hemophilia A due to antibodies of factor VIII procoagulant activity is rare. This paper reports 11 such patients followed up for long periods. They exemplify various forms of associated disorders. Four of them have died from hemorrhages, 4 have had complete remission and 3 are still alive with persistent inhibitors. The inhibitor activity was recovered in the immunoglobulin fraction in all the patients studied. Various forms of treatment were tried but remission related to therapy was seen in only one woman affected post partum. Spontaneous remissions are common and the aim of therapy must be to control acute severe hemorrhage.

Key words: acquired hemophilia, factor VIII antibodies, factor VIII IgG.

Acta Med Scand 208 5 1980

Acquired hemophilia A is a rare defect of blood coagulation due to the development of antibodies to factor VIII procoagulant activity (VIII C). These inhibitors have been referred to as spontaneous to distinguish them from the inhibitors of VIII C which may complicate hemophilia A and which are due to transfusion of blood or factor VIII concentrates.

The first case was reported in 1940 by Lozner et al (14). In 1950 Deutsch (5) published a monograph on the disease and suggested the term "Hemm-körper Hemophilie". Margolius et al (15) gave a classical review of this subject in 1961. Today at least 100 cases are on record (28). Most of them have however been followed up for only a relatively short time.

Patients with spontaneous inhibitors of VIII C have had co-existing established or assumed immunological disorders such as collagen diseases (e.g. lupus erythematosus) (15, 27), drug reactions (e.g. penicillin) (1, 8, 12, 15, 24), certain neoplasms, skin diseases (e.g. pemphigus and psoriasis) (4, 9).

But inhibitors may also develop in women post partum (15, 16, 20, 32) and in otherwise healthy elderly persons (15, 26).

In several respects the bleeding manifestations of acquired VIII C deficiency resemble those in severe hemophilia. The hemorrhages are often life threatening and very difficult to treat since infused factor VIII is rapidly inactivated by the inhibitor. Still a patient may have no significant symptom for weeks or even months despite the persistence of the anticoagulant.

Various forms of treatment have been suggested but as some patients with factor VIII antibodies recover spontaneously it has been very difficult to assess the therapeutic effect of any form of treatment (30).

This paper reports 11 cases of acquired hemophilia seen at our laboratory and followed up for long periods. They exemplify various forms of associated disorders. The inhibitor activity was recovered in the immunoglobulin fraction in all the patients studied. Various forms of therapy were tried but without any demonstrable effect on the inhibitor level.

METHODS AND MATERIALS

Laboratory methods

The factor VIII coagulant activity (VIII C) of plasma and concentrates was assessed from its normalising effect on the recalcification time of platelet rich hemophilia A plasma possessing less than 1% of the normal amount of VIII C. The factor VIII level found was expressed relative to that in a normal standard consisting of pooled plasma from 20 healthy persons (17).

Inhibiting effect of patient's plasma on the recalcification time of normal plasma (simple anticoagulant test).

Abbreviations: VIII C = factor VIII procoagulant activity; VIII:Ag = factor VIII related antigen; APT = activated partial thromboplastin time.

Table I Clinical data on the patients

Cf=cyclophosphamide Az=azathioprine

Case no	Debut	Age at debut (y)	Sex	Associated illness	Therapy	Course
1	1950	42	♀	Dysproteinemia	Steroids no effect	Died after 4 years from bleeding
2	1968	81	♀	Temporal arteritis	None	Died after 2 months from bleeding
3	1956	30	♀	Post partum	Steroids ACTH	Recovery after 8 months in association with ACTH therapy Delivery after 8 years Healthy after 23 years
4	1965	45	♂	Drug reaction (cloxacillin)	Steroids no effect	Died from bleeding after 1 month
5	1975	71	♂	Cancer	F VIII + Cf no effect	Spontaneous remission after 12 months Healthy after 4 years
6	1968	59	♀	None	Az + steroids F VIII + Cf	Alive after 11 years Lasting inhibitor
7	1975	75	♂	None	Cf + Az + steroids transitory effect later no effect	Alive after 4 years Lasting inhibitor
8	1978	72	♂	None (psoriasis)	Cf + steroids	Alive after 1 year Lasting inhibitor
9	1975	61	♀	None (psoriasis)	F VIII + Cf no effect	Spontaneous remission after 3 months No relapse after 4 years
10	1978	80	♀	None (polycythemia)	Cf + steroids	Spontaneous remission during first month No relapse for 1 year
11	1964	79	♂	None	Steroids no effect	Died from bleeding after 1 year

Normal plasma in a volume of 0.2 ml and a similar volume of the plasma to be tested in various dilutions were incubated for 3 min at 37°C after which 0.2 ml 0.03 M CaCl₂ solution was added and the clotting time was determined. The highest dilution of the patient's plasma that prolonged the recalcification time was taken as the anticoagulant titre.

Quantitative determination of factor VIII inhibitor Various dilutions of the plasma to be tested (0.6 ml) were incubated with 0.2 ml of a factor VIII concentrate (3 units of VIII C/ml) at 37°C for 2 hours. As a blank 0.6 ml hemophilia A plasma (less than 1% VIII C and no anticoagulant) was incubated in the same dilutions as the plasma to be tested with 0.2 ml of the factor VIII concen

Table II Results of the coagulation analyses

Case no	Platelets (10 ⁹ /l)	Bleeding time (min)		Coagulation time (min)	APT time (sec)	VIII C (%)	VIII R Ag (%)	P&P (%)	Fibrinogen (g/l)	FV (%)
		Duke	Ivy							
1	165	4		120				80	3.7	
2	186	2	16	130		0		100	3.9	95
3	318	4		178		0		118	4.3	100
4	246	5		105		0		72	6.2	84
5	400	2		30	83	<0.5	196	42	3.6	95
6	206	4		>60	90	<0.5	280	118	4.6	98
7	238				122	<2.5	>400	124	4.3	190
8	200		5	>720	100	<2.5	204	116	3.2	123
9	266		11	125	82	<2.5	210	136	4.8	100
10	800	2	11	40	60	<2.5	280	93	5.2	104
11	338	2		86		0		100	Normal	89
Normal values	125-340	1-5	6-12	6-14	<45	60-160	60-175	80-120	2.0-4.0	80-

trate. Following incubation the blank and the mixtures of plasma and factor VIII were assayed for residual VIII C in the way described above. The inhibitor activity of the plasma was expressed as the number of units of VIII C (1 unit of VIII C is defined as the amount of VIII C present in 1 ml normal plasma) inactivated by 1 ml of the plasma.

Factor VIII related antigen (VIII R Ag) was determined immunologically in the way described by Holmberg and Nilsson (10). The platelet count, bleeding time (Duke and Ivy), coagulation time, activated partial thromboplastin time (APT time), factor XI, factor IX, P&P (prothrombin factors VII and X), factor V and fibrinogen were determined as described elsewhere (17).

Factor VIII concentrates: 1) Human fraction I-O prepared by Kabí (AHF Kabí) according to the glycine method of Blomback and Blomback (3). One bottle (100 ml) contains approximately 300 units of VIII C. 2) Hemate (Hyland method). 4) One ampoule (30 ml) contains about 1 000 units of VIII C.

Protein A Sepharose CL-4B gel was purchased from Pharmacia AB Uppsala, Sweden. Protein A is a cell wall constituent of certain strains of *Staphylococcus aureus*. It selectively binds the Fc fragment of all IgG subclasses except IgG 3 (7, 13).

Purification of IgG containing inhibitor of VIII C on protein A. A column (10×150 mm) containing approximately 5 ml of packed protein A gel was used. To avoid overloading we used 1 ml of the patient's plasma for every ml of the packed gel. After elution of the first peak with 0.5 M NaCl the bound protein was eluted and appeared as a second peak with 0.1 M glycine HCl buffer at pH 2.4.

CASE REPORTS

Clinical data on the patients and results of the coagulation analyses are given in Tables I and II.

Case 1 has been described earlier (21). This was the first case seen in Malmö and was detected as early as 1950. At 9 years of age the patient, a girl, was found to have rheumatic fever. Since the age of 29 she had had a collagen

disease with hyperglobulinemia, continuous fatigue, bilateral pleurisy on several occasions and recurrent transient swellings of the joints, but never with permanent deformities. ESR about 100 mm/h.

For several years she had bruised easily. Bleeding after trauma had always been heavy, but menstruation had been normal. Later subcutaneous hematomas developed spontaneously. The coagulation time had not been measured until 1950 when it proved to be prolonged—25 min (normal range 8–14 min). This prolongation was shown to be caused by a circulating anticoagulant of antihemophilic factor and was present in a titer of 1/10. On paper electrophoresis this anticoagulant was eluted from the gamma globulin fraction.

Despite heavy steroid therapy the coagulation time gradually increased to 120 min. The patient died in 1954 after bleeding from an ovarian cyst with rupture into peritoneal cavity resulting in peritonitis.

Case 2 is an 81-year-old woman with a history of pernicious anemia for 8 years and histologically verified temporal arteritis for 4 years who still required steroids because of the arteritis. Three months before admission spontaneous hematomas and attacks of mucosal bleeding began to occur. Laboratory studies revealed an inhibitor of VIII C in a titer of 1/10. Other coagulation studies, electrophoresis and serological tests revealed nothing remarkable. No therapy was given except steroids in the same dose as she had been receiving when the coagulation defect was detected. The inhibitor remained unchanged. Two months later she died of generalised bleedings.

Case 3 is a 32-year-old woman, has been described earlier (20). Three months after delivery of her first born she had noticed subcutaneous bleedings on her right lower leg without known previous trauma. During the next 5 months painful ecchymotic areas appeared on various parts of the limbs. On admission she was found to have an anticoagulant of VIII C in a titer 1/100. Preparative electrophoresis demonstrated the inhibitor in the gamma globulin fraction.

She received steroids for one month with no demonstrable effect, but a few days after institution of ACTH in a dose of 40 U a day the coagulation time became shorter and the anticoagulant decreased. After one month, 8 months after onset of the subcutaneous bleedings, VIII C was normal.

Eight years later she gave birth to her second child without any complications. She has not had any relapse for over 23 years.

Case 4 was a 45-year-old man with tuberculosis for which he had undergone an operation with segmental resection of the left lung. Postoperative wound infection was treated with chloramphenicol and cloxacillin. An antibiotic reaction with fever, toxicodermia and joint symptoms developed. Five weeks later he had severe bleeding symptoms. The coagulation time was more than 150 min and an anticoagulant of VIII C in a titer of 1/5 was demonstrated. After another month, during which he was given large doses of steroids, he died from abdominal hemorrhage with 7 liters of blood in the peritoneal cavity.

Case 5 was a 71-year-old man who had been operated on for cancer of the large bowel 6 months before admission. He was admitted to hospital with a huge sublingual

Inhibitor titer	Plasma proteins	Serology
U/ml		
10	Hypergamma	AST pos
III	Gamma at lower limit	Normal
100	Normal	Normal
5	Low albumin	AST ASTA pos
1	Infl reaction IgG 26 g/l	Normal
30	Normal	Normal
5	Low haptoglobin	Normal
30	2 small cathodal bands	Normal
5	Normal	ANF pos (1/32)
5	Low haptoglobin	Normal
10	Normal	Normal

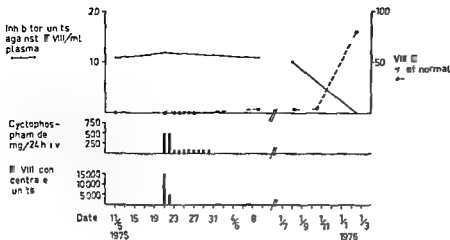


FIG 1 The course in case 5

hematoma and coagulation studies revealed an inhibitor of VIII C in a concentration of 11 U/ml. The inhibitor could be neutralised by anti IgG serum but not by serum against IgM or IgA.

He was treated according to Green (9) with factor VIII (Hematrate) in a dose of 15 300 U and cyclophosphamide 500 mg i.v. on the first day and 5 000 U of factor VIII and 500 mg cyclophosphamide on the second followed by cyclophosphamide 100 mg/day for the next 8 days. However, this treatment failed to neutralise the inhibitor or to raise the VIII C level (Fig. 1). The inhibitor was not affected by this treatment.

Nine months later the inhibitor disappeared spontaneously (Fig. 1) and has not reappeared during the last 4 years.

Case 6 a previously healthy 59-year-old woman has been described earlier (19). In Jan. 1968 she had a depression and was given amitriptyline. In July 1968 subcutaneous bleedings and muscle hematomas appeared without any known previous trauma. Her coagulation time was prolonged and an inhibitor of VIII C was observed in a titer of 1/10. Serological tests as well as electrophoresis revealed nothing remarkable. Large doses of steroids for 12 weeks combined with azathioprine during the last 3 weeks did not result in any improvement.

In 1971 the titer of the inhibitor had risen to 1/20 which corresponded to 162 U/ml. Though inhibitor in such a high concentration cannot be neutralised by factor VIII treatment with cyclophosphamide combined with factor VIII was tried. But her inhibitor concentration remained very high—80 U/ml.

Since 1971 she has not received any therapy. Though she does her utmost to avoid even the slightest trauma, she still has repeated hemorrhages into muscles and joints. When last measured at the end of 1979 the inhibitor was still 150 U/ml.

Case 7 a 75-year-old man who had always felt well. In Jan. 1975 he noticed that he began to bruise readily and had muscle hematomas in his left arm and leg. On admission to his local hospital he was in a very poor condition. His coagulation time was prolonged and he had a circulating anticoagulant. On two occasions he was treated with 500 mg cyclophosphamide i.v. but was not given an factor VIII concentrate. His coagulation time became normal within 14 days.

In Aug. 1976 hemorrhages recurred but this time they were refractory to cyclophosphamide, steroids and azathioprine. He had an inhibitor of VIII C in a concentration of 18 U/ml.

The patient has not had any noteworthy hemorrhage

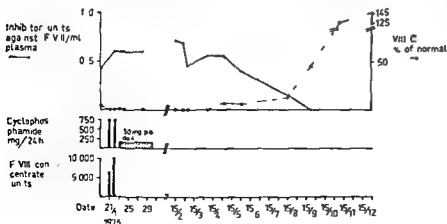


Fig 2 The course in case 9

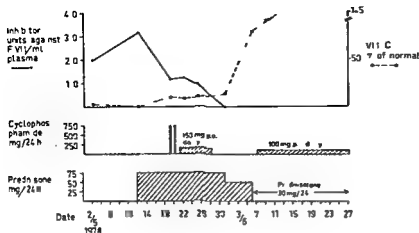


Fig 3 The course in case 10

since then but he still has his coagulation defect. He had no associated illness or drug reaction and electrophoresis has not shown anything remarkable.

Case 8 A 72-year-old man with mild psoriasis and 2-3 years' history of mild arthritis which was thought to be psoriasis arthropathia. In Jan 1978 he noticed that he bruised readily and increased fibrinolysis was suspected at his local hospital. Treatment with Cyclokapron® resulted in a very slow decrease in his bleeding tendency.

Seven months later he developed huge hard hematomas in the right arm, back and legs. Coagulation studies showed an excessively high activity of an inhibitor of VIII C—300 U/ml.

He was treated with cyclophosphamide for 4 months without any demonstrable effect. He has not had any severe bleeding in the last 12 months.

Case 9 A 61-year-old woman who had psoriasis but had always felt well. She complained of pain in her right lower leg and her general practitioner had prescribed oral indomethacin and bandaged her leg. Later removal of the bandage revealed a large hematoma. A few days later she developed hematuria and was admitted to hospital. She proved to have an inhibitor of VIII C—0.5 U/ml.

She was treated with a total dose of 16 300 U of factor VIII and 1 500 mg cyclophosphamide divided between 2 days, followed by cyclophosphamide 150 mg a day (Fig 2). In spite of the large factor VIII dose, VIII C reached only 7.5% immediately after the infusion on day 2 but after 24 hours it was still as high as 3% indicating that the inhibitor was temporarily neutralised. However, after one week the inhibitor was as high as before treatment. Four months later the inhibitor began to decrease and after another 4 months it was no longer demonstrable (Fig 2). She has not had any relapse for over 2 years. Electrophoresis had revealed nothing remarkable. She had a low antinuclear antibody titer viz 1/32.

Case 10 An 80-year-old woman who had passed blood stained stools for two periods in the last year. Roentgenograms of the stomach and intestines were normal. Admitted to hospital with a huge hematoma on her right arm. Coagulation tests showed a low factor VIII—less than 2.5% and an inhibitor of VIII C

The bleeding continued and she was given 8 U blood and steroids. After 2½ weeks treatment with cyclophosphamide combined with steroids was instituted. During the last week before the institution of cyclophosphamide the inhibitor had already fallen from 3.5 to 1 U/ml indicating that cyclophosphamide could hardly have affected the course. The inhibitor disappeared within 3 weeks (Fig 3). She has had no recurrence during 18 months follow-up (18 months) but polycythemia vera has since been diagnosed.

Case 11 was a 79-year-old man who had had a myocardial infarction 12 years earlier. Admitted to hospital with a hematoma in his right arm. Examination at his local hospital revealed prolonged coagulation time. Three weeks later he had several hematomas on the chest and legs and he was given 2 U blood. After transfer to our hospital he was shown to have an inhibitor of VIII C in a titer of 1/10. He was treated with steroids without any demonstrable effect. He died from a bleeding complication one year after the onset of the first symptoms.

RESULTS OF IgG STUDIES IN CASES 6 AND 8

Protein A Sepharose was used in cases 6 and 8 for purification of IgG. In both cases practically all inhibitor activity from the applied plasma was recovered in the eluted IgG fraction.

In case 8 electrophoresis showed 2 very small cathodal bands. IgG in 0.0175 M phosphate buffer pH 6.5 was chromatographed on a DEAE Sephadex A 50 column equilibrated and eluted with the same buffer. On agarose electrophoresis the first portion of the eluate (fractions 15–36) contained the cathodal bands but later fractions eluted with 0.5 M NaCl did not contain these bands. The inhibitor activity of fractions 15–36 was 9 U/mg protein and in

the later fractions eluted with 0.5 M NaCl 15 U/mg protein. This proved that the inhibitor activity was not confined to the cathodal bands.

DISCUSSION

Inhibitors to VIII C have practically always been gammaglobulin immunoglobulins which are not monoclonal. The only known exceptions are one IgA myeloma case and two IgM cases (28).

In cases 1 and 3 of this study preparative electrophoresis showed that the inhibitor could be eluted from the gammaglobulin fraction. In case 5 it was possible to neutralise the inhibitor by an anti-IgG serum. In cases 6 and 8 IgG was purified from plasma and contained all the inhibitor activity. No monoclonal distribution of the inhibitor was found in case 8 in spite of the fact that electrophoresis had shown 2 small M components.

Most spontaneous inhibitors have both κ and λ light chains while inhibitors in hemophiliacs are usually of one light chain type usually κ (28).

Patients with inhibitors of factor VIII procoagulant activity have a severe and often fatal bleeding disorder. In most published cases the course of the disease was not followed up very long. Of our 11 patients 4 died from hemorrhages, 4 had complete remissions and 3 are still alive with persistent inhibitors.

All the patients who died had relatively low inhibitor titers. Two of them died already within the first two months of the onset of their disorder. Steroid therapy had no effect and one of the patients was already receiving steroids when the bleeding disorder started. Two of them had collagen diseases, one had a severe drug reaction and one was an elderly previously healthy man.

Also 3 of 4 patients who had a remission had low inhibitor titers ($\leq 1/10$). Two of these patients had no associated disorder and the third had been operated on for cancer 6 months earlier. In one case the inhibitor had begun to decrease before treatment with cyclophosphamide was started (Fig. 3). In two cases treated according to Green (see below) the inhibitor was not affected. Four and 9 months later respectively the inhibitor disappeared spontaneously (Figs. 1 and 2). In the fourth case the inhibitor appeared post partum. The patient had a titer of 1/100 and the inhibitor disappeared promptly in association with ACTH therapy. In this case we cannot exclude the possibility of a spontaneous re-

mission because the prognosis is relatively favorable and the inhibitor often disappears spontaneously in cases appearing post partum (16, 32). Ten patients with persistent inhibitors are alive 1-4-11 years after onset of the condition. They have the highest inhibitor concentrations and two of them have now inhibitor in an extremely high concentration. None of them have had any associated disorder.

Judging from our maternal patients with a low inhibitor level have a better chance of having remission than those with a high level, but the case mortality is not lower. In fact 6 of 7 patients with low inhibitor levels were either in remission or died one year after the first onset of the symptoms.

Several drugs and treatment regimens have been tried but owing to a variety of associated disorders and the variable course of the inhibitor, caution must be exercised when making any conclusions. It seems important to control an associated disorder (6) and in patients with underlying collagen disorders the inhibitors may disappear on treatment with steroids or other immunosuppressive drugs (27, 33).

In patients without underlying diseases treatment with steroids will usually not affect the inhibitor concentration (11, 15, 34). Treatment with chemotherapeutics alone or combined with steroids has occasionally resulted in complete disappearance of factor VIII antibodies (29, 34) but such therapy is usually of little or no value (9).

Green (9) reported in 1971 a 54-year-old woman with inhibitor of factor VIII. She was initially treated with a combination of methotrexate, azathioprine and cyclophosphamide but without improvement. Rapid administration of 10 000 U of factor VIII in combination with a large dose of cyclophosphamide resulted later in a prompt decrease and disappearance of the inhibitor.

We have not seen any beneficial effect of the different types of therapy except for one woman affected post partum. Our negative results of treatment according to Green might be explained by the fact that in at least two cases we could not infuse sufficient factor VIII to neutralise the inhibitor.

Since one cannot affect the inhibitor level by the different forms of treatment which we have tried, one must anyhow try to achieve hemostasis when a patient has a severe hemorrhage. We are inclined to treat patients with low inhibitor levels in an acute bleeding according to Green with large doses of

factor VIII combined with cyclophosphamide (9). Patients with high levels of inhibitors are almost impossible to treat. However plasmapheresis can lower the inhibitor titer sufficiently to make replacement therapy possible. Such a case has been described by Pintado et al (23). An interesting method is to use for example Aminco plasma pheresis apparatus in combination with large Protein A Sepharose columns. Using this method only the IgG is absorbed and the plasma can be returned to the patient.

Another possibility might be treatment with activated factor IX. Various factor IX preparations can reportedly secure hemostasis in hemophilic patients with inhibitors of factor VIII (2, 25). Nevertheless Parry and Bloom (22) did not find recently any therapeutic effect of FEIBA (Immuno Austria) on 14 separate bleeding episodes.

ACKNOWLEDGEMENT

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Lymphocytopenia Preceding Chronic Lymphocytic Leukemia

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ABSTRACT In 11 patients with an average peripheral blood lymphocyte count of $21.1 \times 10^9/l$ at the time of diagnosis of chronic lymphocytic leukemia (CLL) WBC and differential counts had been recorded on 1-26 occasions more than 5 years before the diagnosis. The median lymphocyte count in the preleukemic period was $1.3 \times 10^9/l$, which is significantly lower than $2.4 \times 10^9/l$ found in a sex and age-matched control series. In 3 patients lymphocytopenia coexisted with normal levels of serum immunoglobulins in the preleukemic period. It is suggested that the preleukemic lymphocytopenia was mainly due to low numbers of T cells and that a deficiency in T lymphocytes may favour the development of the malignant, monoclonal B-cell proliferation characteristic of CLL.

Key words: chronic lymphocytic leukemia, preleukemia, lymphocytopenia.

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The mechanisms behind the development of chronic lymphocytic leukemia (CLL) remain unclear (9). Attempts to analyse the preleukemic period might help to elucidate the pathogenesis of the disease.

Recently we made a diagnosis of CLL in an elderly woman who had been hospitalized several times during the last 44 years because of diabetes mellitus. According to her case records strikingly low lymphocyte count had been recorded repeatedly before the diagnosis of CLL. We therefore decided to investigate whether lymphocytopenia may be a common phenomenon preceding the lymphocytosis characteristic of CLL.

PATIENTS AND METHODS

Patients under observation or treatment for CLL at our departments were interviewed about previous medical examinations and efforts were made to obtain the relevant case records. In 11 patients 4 female and 7 male

aged 38-88 years and with lymphocyte counts of $10.6-31.3 \times 10^9/l$ (median 21.1) at the time of diagnosis information was obtained on WBC and differential counts 5-44 years before the diagnosis of CLL. The disorders requiring medical attendance in the preleukemic period are given in Table I. The lymphocyte counts were calculated from the WBC and differential counts. Lymphocyte counts recorded during treatment with corticosteroids were omitted. In 3 patients the serum gammaglobulin concentration had been determined together with WBC and differential counts 6, 10 and 12 years before the diagnosis of CLL.

A control group was obtained through assessment of the lymphocyte counts in a series of consecutive patients investigated for mainly cardiac complaints in whom acute myocardial injury or other serious diseases could be excluded with reasonable certainty. Two controls matched for sex and age were selected for each CLL patient studied in the preleukemic phase.

RESULTS

WBC and differential counts had been recorded more than 5 years before the diagnosis of CLL in 11 patients. Two or more observations were available in 6 patients (nos. 1-6) in 5 (nos. 7-11) the analysis had been performed only once (Figs. 1 and 2). Lymphocyte counts below $1.5 \times 10^9/l$ had been recorded in the preleukemic period in 7 (64%) of the 11 patients. Only 3 (14%) of the 22 controls had counts below this level.

In Fig. 3 the preleukemic lymphocyte counts are compared with the counts in the controls. In this comparison the median value of the lymphocyte counts in each of the patients examined more than once was considered to represent the number of lymphocytes in the preleukemic period. The median preleukemic lymphocyte count was $1.3 \times 10^9/l$ which is significantly lower than the median value of $2.4 \times 10^9/l$ found in the control group ($p < 0.002$, Wilcoxon test).

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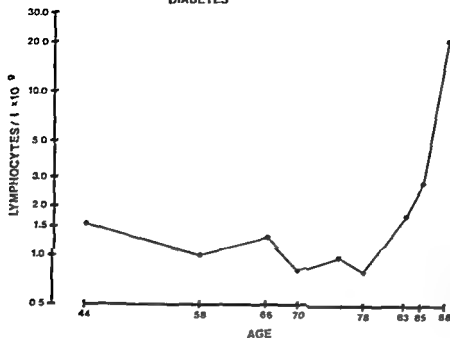
CASE 1 ♀
DIABETES

Fig 1 Lymphocyte counts before and at the time of diagnosis of CLL in a woman with diabetes followed for 44 years

In 3 patients gammaglobulin concentrations were assessed in the preleukemic period. All had normal levels coexistent with lymphocytopenia (Table II).

DISCUSSION

The decreasing lymphocyte counts in case 1 (Fig 1) observed during a period of more than 30 years suggest that a reduction of the peripheral blood lymphocytes may precede the development of CLL. The findings in the total series more than 5 years before the diagnosis of CLL indicate that

lymphocytopenia may be common in the preleukemic phase of CLL.

The present retrospective study cannot show whether the low lymphocyte counts were due to a deficiency in any special subpopulation of lymphocytes. In the controls the average lymphocyte count was $2.4 \times 10^9/l$. In the peripheral blood of healthy subjects the number of immunoglobulin bearing lymphocytes (i.e. B lymphocytes) is about $0.2-0.7 \times 10^9/l$ (6). The average preleukemic lymphocyte count of $1.3 \times 10^9/l$ found in our patients is therefore hardly compatible with a selective deficiency in B-cells. Moreover the findings of normal levels of

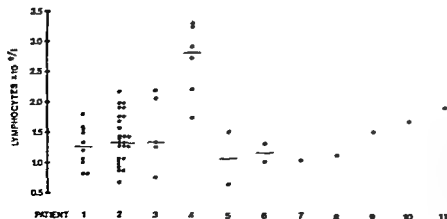


Fig 2 Preleukemic lymphocyte counts in patients 1-11. The median is indicated for patients with two or more assays

Table I Clinical data on 11 patients examined more than 5 years before the diagnosis of CLL

Patient no	Sex	Disease in pre-leukemic period	Period studied before diagnosis of CLL (y)	Age at diagnosis of CLL (y)	Lymphocytes at diagnosis of CLL ($\times 10^9/l$)
1	♀	Diabetes	5-44	88	21.5
2	♀	Discoid lupus	8-22	81	31.3
3	♂	Diabetes	12-14	70	13.4
4	♂	Diarrhoea cause unknown	7-24	56	25.5
5	♂	Pneumonia	6	57	21.7
6	♂	Temporal arteritis	10	72	18.4
7	♂	Bronchial asthma	7	62	10.7
8	♂	Duodenal ulcer	5	57	20.1
9	♂	Multiple sclerosis	7	38	20.2
10	♀	Diabetes	10	77	21.6
11	♀	Suspected but not verified pericardial cyst	11	76	21.1

serum immunoglobulins associated with lymphocytopenia suggest that the B lymphocyte functions were intact in the preleukemic lymphocytopenic phase. It is therefore probable that the low lymphocyte counts were mainly due to a deficiency in T-cells.

Although CLL is usually a B cell disorder of monoclonal origin (2, 4), increased numbers of T lymphocytes have been demonstrated. Catovsky et al. (3) found that in a group of untreated stable CLL patients the number of T lymphocytes in peripheral blood was on an average $3.6 \times 10^9/l$, i.e. considerably higher than the lymphocyte counts recorded in the preleukemic phase of the present patients. A minute T cell population in the preleukemic phase of CLL may therefore be considerably increased, possibly as a reaction to the establishment of a malignant clone of B lymphocytes.

There is experimental evidence that thymus-derived suppressor cells can restrict the proliferation

of B lymphocytes and that an impaired T cell regulation of B cell proliferation may favour the development of autoimmunity and lymphoproliferative disorders (5). It has been suggested that in humans a defective T cell activity may be associated with the development of B cell neoplasms in patients infected with EB virus (8). Pronounced antigenic stimulation of B lymphocytes in patients with an impaired suppressor cell function may likewise promote the development of a lymphoproliferative disorder (7). It is therefore possible that viral or other stimuli causing an increased proliferation of B lymphocytes in a situation with co-existing deficiency in T-cells may increase the risk of development of a malignant clone of B-cells.

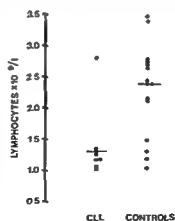


Fig. 3 Lymphocyte counts in the controls and in the preleukemic period of 11 CLL patients. The median is indicated for patients with two or more preleukemic counts.

Table II Immunoglobulin concentrations and lymphocyte counts in 3 patients 6-12 years before diagnosis of CLL

	Immunoglobulin concentrations	Normal values	Lymphocyte count ($\times 10^9/l$)
Case 1	γ globulin 11.2 g/l	6.1-11.7	10
Case 6	γ globulin 12.3 g/l	6.1-11.7	10
Case 7	IgG 70%	65-160	11
	IgA 115%	50-160	
	IgM 155%	30-200	
	(% of normal serum pool)		

Lymphocytopenia i.e. lymphocyte counts lower than $1.5 \times 10^9/l$ (10) was found in 14% of the controls indicating that lymphocytopenia is not uncommon in the general population. CLL is a relatively rare disease and factors in addition to lymphocytopenia are therefore obviously necessary to bring about the malignant proliferation. It may be that a deficiency in T-cells constitutes a risk factor for CLL. Such a concept is supported by the facts that ageing is characteristically associated with a decrease in T lymphocytes (1) and that CLL patients are often old.

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Blood Pressure and Renal Function

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ABSTRACT The relationship between blood pressure (BP) and renal function was studied in samples of 49 year old men. Of 3205 49 year old men, 2376 (74%) took part in a BP screening. By systematic sampling, based on diastolic BP levels varying from very low to very high, 120 subjects were selected for this study. Only subjects who were not on antihypertensive treatment were included. Renal blood flow (RBF), renovascular resistance (RVR), glomerular filtration rate (GFR), filtration fraction (FF) and renal concentrating capacity were studied in 111 subjects, none of whom had advanced hypertension. With increasing BP there was a decrease in RBF ($r = -0.34$) and an increase in RVR ($r = 0.81$) and FF ($r = 0.35$). The changes in renal haemodynamics occurred gradually from low to high BP, and did not start at any particular BP level. With increasing BP, GFR was unchanged. An "autoregulation of GFR" was thus found at all BP levels studied. Renal concentrating capacity was unchanged. These findings indicate that renal haemodynamics in essential hypertension are adjusted mainly to ensure a constant GFR.

Key words: essential hypertension, glomerular filtration rate, renal blood flow, renal vascular resistance, kidney concentrating ability, epidemiology.

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In 1938 Smith et al. (39) presented the first study on renal function in essential hypertension in man using the clearance technique. They found that renal blood flow (RBF) was less and the filtration fraction (FF) greater in hypertensive than in normal subjects. Most of the hypertensive subjects had a normal glomerular filtration rate (GFR). In later studies, varying findings have been obtained concerning RBF, GFR and FF in essential hypertension. Most studies have shown a decrease in RBF in all stages of the hypertensive disease (6, 11, 23) but in some studies RBF has been unchanged in early

hypertension (16, 24). In some studies GFR has been within the normal ranges (7, 11, 16) but a significant decrease also in GFR has been found in others. However, GFR has been less reduced than RBF, leading to an increased FF.

Maximal renal concentrating capacity in essential hypertension has been studied less. Some investigators have found a marked decrease in concentrating capacity early in the hypertensive disease (15, 30), whereas others (20) have found it normal until late in the disease.

Thus, the results regarding renal function in essential hypertension have been conflicting, especially concerning the early stage of the disease. The discrepancy in results has in many instances been due to differences in selection of patients, especially with regard to duration and severity of the hypertension. We have studied the renal function in an age homogeneous group of subjects in an attempt to define the functional changes in the earliest stages of the hypertensive disease.

STUDY POPULATION

In order to find 120 subjects of the same age representing the entire range of blood pressure (BP) levels in the population, every second 49 year old male resident in Göteborg (i.e. born 1926-27) was invited to participate in a screening examination of BP.

The expected number of individuals in various diastolic BP (DBP) groups was calculated from the BP distributions

Abbreviations: RBF = renal blood flow, FF = filtration fraction, GFR = glomerular filtration rate, BP = blood pressure, DBP = diastolic BP, SBP = systolic BP, MAP = mean arterial pressure, BSA = body surface area, PAH = p-aminohippurate, Cl_{PAH} = PAH clearance, Cl_{inulin} = inulin clearance, $Cl_{creatinine}$ = creatinine clearance, Hct = haematocrit, RVR = renovascular resistance.

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Table 1 Plan for selection of subsamples from the 49 year old male population with regard to DBP and date of birth

DBP at screening (mmHg)	Date of birth	Proportion of the population	Expected no of untreated subjects
<95	18	1/30	25
95-104	8 18	1/15	15
105-114	8 12 18 26 30	1/6	15
≥115	1-31	1/1	65

of earlier screening examinations of middle aged men in Göteborg (9). The selection plan included 1/30 of the subjects with a DBP at screening <95 mmHg, 1/15 of those with DBP 95-104 mmHg, 1/6 of those with DBP 105-114 mmHg, and all subjects with DBP ≥115 mmHg. This was achieved by systematic sampling based on the subjects' date of birth (Table 1). The days 8, 12, 18, 26 and 30 were taken from a table of random numbers. Subjects who met the criteria and were not on antihypertensive treatment were asked to participate in the investigation.

The 1926 and 1927 age groups together consisted of 5336 men. When the planned number of men had been screened, we found fewer subjects with DBP ≥115 mmHg than expected. An additional number of men born in 1927 were therefore screened. Altogether 59.7% ($n=3205$) of the two age groups (50% and 70.2% respectively) were invited to the screening. 74.1% ($n=2376$) of the invited subjects attended. A total number of 107 (4.5%) subjects were on antihypertensive treatment. 179 fulfilled the selection criteria but 36 of them were on antihypertensive treatment and were therefore not included in the study. Another 8 were excluded because of diseases (valvular heart disease, 4; previous myocardial infarction, 1; previous stroke, 1; chronic glomerulonephritis, 1; and severe chronic alcoholism, 1) and 15 subjects refused to participate in further investigations. The remaining 120 subjects took part in the investigation. They comprised 9% (120/135) of those who were eligible for the study. In addition to the renal function studies, an investigation of heart function and metabolic and hormonal variables was performed and will be reported separately.

METHODS

The investigations took place at Sahlgrenska Hospital. Screening was performed in 1975 and 1976 from March to May and from Sept to Nov, between 4.30 and 7.00 p.m. The renal function tests were carried out within one month of screening and were followed by a diagnostic work-up at the Hypertension Clinic. The subjects were on unrestricted diet and were treated as outpatients throughout the study.

Blood pressure

BP was measured in four different situations. The arithmetic mean of the BP recordings in each situation was used. DBP was recorded at phase five. Mean arterial BP (MAP) was calculated as $DBP + \frac{1}{3}$ of the pulse pressure. The rubber cuff used at screening was 12×30 mm and this was used in the other three situations 12×35 mm.

Casual BP. At screening, BP was measured once in the right arm with the subject in the seated position after a few minutes' rest with a mercury manometer by the auscultation method. BP was determined in the nearest 2 mmHg, in order to avoid digital preference.

BP during the clearance procedure. determined to the nearest 1 mmHg, was measured in the right arm with the subject in a semirecumbent position with an automatic device for cuff inflation and deflation with simultaneous registration of cuff pressure, Korotkoff sounds and ECG (44). During the clearance periods, BP was registered twice every 10 min, altogether 18 times during two hours. The mean of these BP values was used.

BP after rest. was measured twice after the subjects had been resting recumbent for 45 minutes in a sound protected room. The same technique as at the clearance investigation was used.

BP at the Out Patient Hypertension Clinic. was measured on three different occasions within one month of the renal function studies. BP was registered after 5 min supine rest, using the same method as at screening.

Heart rate

At screening and at the Hypertension Clinic, heart rate was determined by pulse palpation in the clearance periods and after 45 min of rest it was calculated from the BP recordings.

Height and weight

The method of Rose and Blackburn (36) was applied to these measurements. Body surface area (BSA, m²) was calculated according to Isaksson's method (27):

$$1 + \frac{\text{height (cm)} - 160 + \text{weight (kg)}}{100}$$

Clearance

GFR was measured as inulin clearance (C_{I_{in}}) and renal plasma flow as p-aminohippurate clearance (C_{I_{PAH}}) using the continuous infusion technique. Extraction of PAH was not measured. The clearance measurements were performed between 8 and 11 a.m. After a priming dose of 4 ml of 25% inulin (Inutest, Laevosan) and 20 ml of 20% PAH (Sodium Aminohippurate, MSD) dissolved in 100 ml of saline (0.9%), a constant infusion was given, aiming at a plasma concentration of 20 mg/100 ml for inulin and 2 mg/100 ml for PAH. After an equilibrium period of 45 min, three consecutive 40-minute clearance periods were studied. The subjects were hydrated with 10 ml of water/kg wt before the clearance measurements and water was then given to compensate for diuresis. The diuresis was 8.2±3.7 ml/min (mean ± SD) during the three clearance periods. Urine was collected without catheterization. A plastic cannula was inserted into a cubital vein in each

arm. The infusion was given in the left arm and blood samples were taken from the right arm in the middle of each period. Inulin was analyzed according to the method of Hubbard and Loomis (25) and PAH according to the method of Brun (13). The errors of inulin and PAH measurements were checked in a series of 20 consecutive samples which were analyzed twice. The difference between the first and second determination was not significant. The error of a single determination expressed as per cent of the mean was 4.5% for u-inulin, 9.1% for p-inulin, 5.6% for u-PAH and 5.0% for p-PAH. The values of Cl_i and Cl_{PAH} were corrected for 1.73 m² BSA. Clearance measurements were successfully performed in 111 of the 120 subjects. The reason for failure was difficulty in voiding in 8 cases and a pronounced vasovagal reaction in one case. Owing to voiding difficulties and technical problems 13 of the 111 men were subjected to the test a second time within one month.

Endogenous creatinine clearance (Cl_c) was determined from the 24-hour urinary creatinine excretion rate and the plasma creatinine concentration. Urine was collected by the subjects for three consecutive 24-hour periods after giving them detailed instructions. Blood for p-creatinine was drawn in the morning after the last 24-hour urinary collection had been completed. Creatinine was determined by Autoanalyser at the Department of Clinical Chemistry. The mean Cl_c of the three 24-hour periods was used. The value was corrected to 1.73 m² BSA.

Filtration fraction was calculated as the Cl_i/Cl_{PAH} ratio. **Haematocrit (Hct)** was measured after centrifugation of blood in a Hct-centrifuge (Kemila, Sweden).

Renal blood flow was calculated as $Cl_{PAH}/1.1 \text{ Hct}$ (38).

Renal vascular resistance (RVR) was calculated as

$$\frac{\text{MAP during clearance (mmHg} \times \text{min)}}{\text{RBF}} = \frac{\text{mmHg} \times \text{min}}{\text{ml} \times \frac{\text{min}}{\text{cm}^3}} = \frac{\text{mmHg} \times \text{min}}{\text{cm}^3} \times \frac{1}{\text{min}} = \frac{\text{mmHg}}{\text{cm}^3}$$

$$\frac{8.0 \times 10^4 \text{ Pascal} \times \text{s}}{\text{m}^3}$$

Renal concentrating capacity was measured using the vasopressin test (Pitressin tannate in oil, Parke Davis) according to the method of de Wardener (42).

Diagnostic work up

A detailed history was recorded and a physical examination was performed at the Hypertension Clinic. The ocular fundi were examined. A chest X-ray was taken and the serum concentrations of sodium, potassium, calcium, creatinine, urate and plasma aldosterone were measured. Urine tests for glucose, sediment and bacterial culture were also performed. The 24-hour urinary excretion of albumin, catecholamines and cortisol was determined. Isotope renography was performed using standard methods and equipment (2). In patients with abnormal radiorenograms further investigation was performed by pyelography or renal aortography.

Characteristics of the study population (n=111)

Three clinical groups were defined mainly to make comparisons with other studies possible. Subjects with a DBP of ≥ 105 mmHg (mean of three BP measurements) at the

Out Patient Hypertension Clinic were regarded as hypertensives (n=23). Subjects with a screening DBP of <95 mmHg and a DBP of ≤ 90 mmHg (mean of three recordings) at the Hypertension Clinic constituted the normotensive group (n=20) and the remaining subjects were allocated to the borderline group (n=68).

Height, weight and BSA in the three groups are shown in Table II. Both the borderline subjects and the hypertensives were significantly heavier than the normotensives and had a larger BSA. Mean weight was 10.4 kg higher in the hypertensives than in the normotension group.

The prevalence of cardiac enlargement ($\geq 500 \text{ ml/m}^2$ BSA) left ventricular hypertrophy on the ECG, elevated s-creatinine and increased urinary albumin excretion is shown in Table III. In the hypertension group three of the subjects with cardiac enlargement on X-ray also had left ventricular hypertrophy on ECG. Heart size was calculated according to the method of Jonell (28). Left ventricular hypertrophy on the ECG was diagnosed according to the Minnesota Code (3, 1 or 3, 3 plus 4, 1-3 or 5, 1-3) i.e. a combination of amplitude and ST or T criteria was used (36).

Fundoscopic changes were all of grade I-II. None of the subjects had a history of or showed signs of myocardial infarction, angina pectoris, cardiac decompensation, intermittent claudication, stroke or diabetes mellitus. Abnormal renal findings were seen in only 4 subjects. One non-hypertensive subject had unilateral kidney aplasia with contralateral kidney hypertrophy (16x7.5 cm). His Cl_i was 101 and Cl_{PAH} 574 ml/min. Two hypertensive subjects had slight proteinuria ($<1 \text{ g/24 h}$) but no haematuria. They had a GFR of 110 and 110 ml/min respectively and had no history of chronic glomerulonephritis. Another hypertensive subject with constant microscopic haematuria had chronic prostatitis diagnosed by cystoscopy. He had no proteinuria and his Cl_i was 95 and Cl_{PAH} 602 ml/min. All three hypertensives had normal pyelography findings.

Statistical methods

Standard methods were used for calculation of means, S.D., variation coefficients and correlation coefficients. The hypothesis of no difference in means between two groups was tested by Student's *t* test. Only two-sided tests were used. The hypothesis of non-linear correlation between two variables was tested using the correlation coefficient. Values of $p < 0.05$ were regarded as statistically significant.

In addition the sliding mean value method (14) was used to describe the relationship between mean arterial BP during the clearance measurements and the renal function variables in the following way. The subjects studied were ranked from 1 to 111 by increasing BP and then subgrouped. The first subgroup comprised subjects with the lowest BP (nos 1-40). The next subgroup comprised subjects 2-41, the third subjects 3-42 and so on. The mean value of BP and the mean value of the studied variable were plotted for each subgroup. The points were then joined to form continuous curves. The mean values varied very gradually since one subgroup differed from the next by only two subjects. Two extra subgroups comprising

Table II Systolic and diastolic BP (SBP DBP) mean arterial blood pressure (MAP) and heart rate (HR) in four different situations and height weight and body surface area (BSA) in the clinical groups (mean S D)

	Normotension group (a) (n=20)	Borderline group (b) (n=68)	Hypertension group (c) (n=23)	t tests for a b c (p<)		
				a b	a c	b c
At screening						
SBP	130±11	162±14	189±16	0.001	0.001	0.001
DBP	84±7	113±10	128±12	0.001	0.001	0.001
MAP	99±8	130±11	148±12	0.001	0.001	0.001
HR	76±14	82±13	86±12	n.s.	0.025	n.s.
At the Hypertension Clinic						
SBP	127±7	146±11	176±16	0.001	0.001	0.001
DBP	76±6	93±8	115±7	0.001	0.001	0.001
MAP	93±5	111±9	135±9	0.001	0.001	0.001
HR	70±11	74±9	77±13	n.s.	n.s.	n.s.
During clearance measurements						
SBP	115±12	134±13	162±24	0.001	0.001	0.001
DBP	70±7	86±9	103±15	0.001	0.001	0.001
MAP	85±7	101±10	123±17	0.001	0.001	0.001
HR	64±10	70±11	73±11	0.05	0.01	n.s.
After 45 min rest in a sound protected room						
SBP	112±11	132±13	158±24	0.001	0.001	0.001
DBP	63±9	80±11	97±13	0.001	0.001	0.001
MAP	80±8	97±11	117±16	0.001	0.001	0.001
HR	62±10	67±11	69±8	n.s.	n.s.	n.s.
Height (cm)	176.4±5.2	177.0±7.0	176.2±7.0	n.s.	n.s.	n.s.
Weight (kg)	75.2±8.7	82.6±12.2	85.6±13.8	0.005	0.01	n.s.
BSA (m ²)	1.92±0.11	2.00±0.17	2.02±0.18	0.02	0.05	n.s.

subjects 1-20 and 92-111 were formed in order to study the extreme values. These curves thus describe how the mean value of a variable on the y axis changes when BP on the x axis increases. The correlation between the variables studied is not demonstrated by these curves and is expressed in the usual way i.e. by the correlation coefficient and the regression equation. The calculations were made by a computer (IBM 360/65) or a desk computer (P 652 Olivetti).

RESULTS

Blood pressure and heart rate

Table II gives the BPs and heart rates in the four different situations for the three clinical groups. The values were significantly higher in screening than at the Hypertension Clinic except for SBP and heart rate in the normotension group. Of the 6

Table III Prevalence of cardiac enlargement ECG abnormalities elevation of s creatinine and urinary albumin excretion in the three clinical groups

	Normotension group (n=20)		Borderline group (n=68)		Hypertension group (n=23)	
	n	%	n	%	n	%
Cardiac enlargement on X ray (>500 ml/m ² BSA)	1	5.0	3	4.4	4	17.4
Left ventricular hypertrophy on ECG	0	0	4	5.9	6	26.1
S-creatinine >114 µmol/l (>1.3 mg/100 ml)	0	0	0	0	0	0
U albumin >200 mg/24 h	11	55	2	2.9	4	17.4

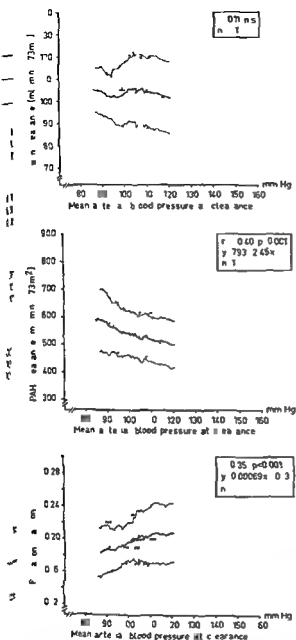


Fig 1 Mean values (thick lines) and S D (thin lines) for Cl_{Cr} , Cl_{PAH} and Cl_{in} in the successive BP groups. The dashed lines connect the means of the small groups ($n=20$) with the lowest and highest BP with the nearest group of 40 subjects. The result of linear regression analysis is also given.

subjects having a DBP ≥ 115 mmHg at screening only 22 (32.8%) were hypertensive according to our arbitrary definition. BP during the clearance periods was almost as low as after 45 min rest in the sound-protected room, which indicates that the clearance

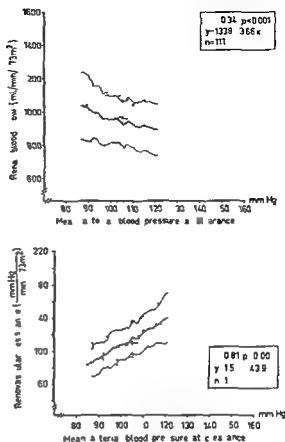


Fig 2 Mean values and S D for RBF and RVR in the successive BP groups. For explanation of the figure see text to Fig 1.

measurements were performed under almost basal conditions.

Inulin clearance

Cl_{in} did not increase significantly with increasing BP ($r=0.11$) (Fig 1). There was no significant difference in Cl_{in} between the three clinical groups (Table IV).

Endogenous creatinine clearance

Cl_{Cr} did not change significantly with increasing BP ($r=0.16$) and did not differ between the three clinical groups (Table IV). The S D of Cl_{Cr} constituted 71–75% of the mean value and that of Cl_{in} 10–14% in the three clinical groups. The mean Cl_{Cr}/Cl_{in} ratio was 1.3.

PAH clearance

Cl_{PAH} decreased significantly with increasing BP ($r=0.40$, $p<0.001$) (Fig 1). The decrease was

Table IV Inulin clearance (Cl_{in}) endogenous creatinine clearance (Cl_{cr}) PAH clearance (Cl_{PAH}) filtration fraction (FF) haematocrit (Hct) renal blood flow (RBF) renovascular resistance (RVR) and urine concentration test in the normotension borderline and hypertension groups (mean \pm S D)

Cl_{in} Cl_{cr} Cl_{PAH} RBF and RVR are corrected to 1.73 m² BSA

	Normotension group (a) (n=20)	Borderline group (b) (n=68)	Hypertension group (c) (n=23)	t tests for a b c (p<)		
				a b	a c	b c
Cl_{in} (ml/min)	108.8 \pm 10.5	103.3 \pm 13.9	102.2 \pm 14.2	n.s.	n.s.	n.s.
Cl_{cr} (ml/min)	133.9 \pm 28.6	131.3 \pm 30.2	128.2 \pm 31.9	n.s.	n.s.	n.s.
Cl_{PAH} (ml/min)	616 \pm 123	532 \pm 87	505 \pm 91	0.01	0.005	n.s.
FF	0.184 \pm 0.035	0.197 \pm 0.031	0.207 \pm 0.029	n.s.	0.05	n.s.
Hct (%)	42.8 \pm 1.9	43.5 \pm 2.1	44.7 \pm 2.2	n.s.	0.005	0.05
RBF (ml/min)	1088 \pm 221	942 \pm 152	911 \pm 150	0.02	0.005	n.s.
RVR (mmHg/1/min)	82.0 \pm 17	111.2 \pm 23	139.8 \pm 36	0.001	0.001	0.005
Urine concentration test (mOsm/kg H ₂ O)	995 \pm 118	979 \pm 107	974 \pm 130	n.s.	n.s.	n.s.

gradual and continuous Cl_{PAH} was also significantly lower in the borderline and hypertension groups than in the normotension group but there was no significant difference between the borderline and hypertension groups (Table IV)

Filtration fraction

FF increased significantly and linearly with increasing MAP ($r=0.35$ $p<0.001$) (Fig. 1) FF was significantly higher in the hypertensive than in the normotension group but there was no significant difference in FF between the borderline group and the other groups

Haematocrit

Hct increased significantly with increasing MAP ($r=0.37$ $p<0.001$) Hct was significantly increased in the hypertension group compared with the other two groups but there was no significant difference in Hct between the normotension and the borderline group (Table IV)

Renal blood flow and renovascular resistance

As anticipated from the Cl_{PAH} curve in Fig. 1 RBF decreased significantly with increasing BP ($r=-0.34$ $p<0.001$) (Fig. 2) The decrease was continuous and started in the lowest BP range. The decrease with increasing BP in RBF was less steep than in Cl_{PAH} owing to the increase in Hct with increasing MAP. RBF was significantly lower in the borderline and hypertension groups than in the normotension group but there was no significant difference in RBF between the borderline and the hypertension groups (Table IV). The finding that

RBF decreased with increasing BP means that RV increased drastically with increasing BP ($r=0.8$ $p<0.001$). The relationship seems to be linear. The difference in RVR between any two of the clinical groups was also significant (Table IV).

Renal concentrating capacity

Urine osmolality in the whole study group did not change significantly with increasing BP ($r=0.01$) but there was a tendency towards higher concentrating capacity in the lowest limits of the BP range (Fig. 3). There was no significant difference in urine osmolality between the three clinical groups (Table IV).

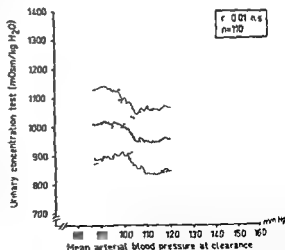


Fig. 3 Mean values and S D for maximal urinary concentrating capacity in the successive BP groups. For explanation of the figure see text to Fig. 1

DISCUSSION

This study of renal haemodynamics and renal function in essential hypertension was performed in a nonselected population sample of untreated 49 year old men. The results are not influenced by variations due to age, sex or treatment, which is important since RBF, GFR and renal concentrating capacity are known to decrease with age (17-34) and may be lower in women than in men (38-43) and since antihypertensive treatment even after withdrawal for several weeks (3) is known to influence many of the variables studied (18-26, 35).

An enrichment of subjects in the upper part of the BP range was performed in order to obtain a sufficient number of subjects to ensure a correct description of factors related to BP in the borderline and definitely hypertensive part of the BP distribution. The criteria for inclusion should have ensured that these subjects were representative for all BP levels. Thus the analysis provides an opportunity to determine at what BP level and in what sequence various functional changes appear.

Although BP is roughly normally distributed in the population—which means that there are no fixed limits for normotension, borderline or definite hypertension—three such groups were defined to allow comparison with other studies and to give absolute values for these groups mainly defined according to clinical criteria established in Sweden (8). However it is difficult to compare the absolute values in this study with those in some other studies of renal function in selected groups with varying age, sex and severity of hypertension. This is exemplified by a recent cross sectional study in which renal function in hypertensives was related to age in order to study the changes in renal function in relation to the duration and development of hypertension (29). 207 subjects with BP higher than 150/100 mmHg (age range 17-63 years) were studied. An increase in BP and a decrease in GFR and renal plasma flow with increasing age were found. Renal plasma flow decreased more steeply with age in these hypertensives than in normal subjects in various other studies, but it is difficult to determine how much of the observed changes were caused by aging and how much by hypertension.

The finding in our study that GFR did not differ significantly between normotensives and untreated hypertensives is in accordance with the results of an earlier study of a random sample of middle aged

men in whom GFR was measured by a single injection technique (7). In that study GFR was 100 ml/min in normotensives and 96 ml/min in hypertensives. These values are slightly lower than those found by us, but the single injection technique gives slightly lower GFR than inulin clearance (12).

The decrease in RBF with increasing BP may be an effect of both the severity and the duration of elevated BP. The steeper decrease in renal plasma flow than in RBF was due to an increase in Hct with increasing BP. Higher Hct values in hypertensives than in normotensives have previously been reported in a random population sample (40). The absolute values of RBF are underestimated as no correction for PAH extraction was made. According to Smith (38) the PAH extraction is about 0.90 in normal subjects and it is not substantially reduced until Cl_{PAH} is less than 300 ml/min. As no subject in the study had a renal blood flow of less than 300 ml/min or abnormally low GFR, it is likely that the PAH extraction was the same at all BP levels.

The decrease in RBF with increasing BP may be explained by increased sympathetic discharge to the kidneys and/or the action of circulating vasoconstrictors. The renal sympathetic activity recorded from single fibres is reported to be twice as high in spontaneously hypertensive than normotensive rats (41). The question whether there is an increased activity of the sympathetic nervous system in essential hypertension has not been settled (4, 32, 37). The response to both low doses of noradrenaline and angiotensin II closely mirrors the haemodynamic pattern seen in this study including largely unchanged GFR, decreased RBF and increased FF (1, 21, 22). The decrease in RBF can also be explained by structural changes in the renal resistance vessels in the form of an increased media thickness leading to an increased wall/lumen ratio. Such changes have been shown to give rise to an exaggerated resistance response of the renal vascular bed to graded doses of noradrenaline in spontaneously hypertensive rats compared with normotensive controls, although the sensitivity to threshold doses of noradrenaline was the same in the two types of rats (19).

Thus the study has shown that the primary feature in the early stages of essential hypertension is an adjustment of renal haemodynamics so that the glomerular filtration is kept constant. The adjustment is accompanied by an increase in FF. This

indicates an increased efferent to afferent arteriolar resistance. The filtration pressure in the glomerular capillary will thereby be maintained or even slightly increased. Direct measurements with micro-puncture technique have shown higher glomerular capillary pressures in certain strains of hypertensive rats than in controls (5).

GFR is also determined by the area and the permeability of the glomerular capillary. There is no evidence that an increase of the area would be present in early hypertension. A positive correlation has been found between BP and urinary albumin excretion both in normotension and hypertension (33) indicating a significant influence of the BP on the permeability of the glomerular capillary for macromolecules. It has been suggested that the permeability of the glomerular capillary may be decreased in hypertension owing to a genetic defect (10). This defect would be compensated by an increase in the filtration pressure.

The intrarenal pressure may also influence the filtration pressure and hence the GFR. Lowenstein et al (31) observed an increase in intrarenal pressure in hypertensive subjects compared to normotensives but this could not be reproduced by Willassen and Ofstad (45). If the intrarenal pressure would be elevated it would tend to decrease GFR which is not consistent with our findings. The available evidence thus indicates that the net filtration pressure is slightly increased in early hypertension maintaining the GFR despite the decrease in RBF.

The renal concentrating capacity was not significantly correlated to the BP levels in our subjects all of whom had normal or slightly reduced GFR. We therefore find it highly improbable that the concentrating capacity is reduced early in the hypertensive disease as has been claimed (30).

From this study in an epidemiologically defined population based on 111 persons we conclude that renal function in early stages of essential hypertension is characterized by 1) Increased renovascular resistance and decreased RBF. These haemodynamic changes do not start at any particular BP level. 2) Unchanged GFR accompanied by an increase in FF. Thus an autoregulation of GFR was demonstrated at all BP levels studied. The evidence suggests that GFR is maintained by an increase in glomerular capillary pressure. 3) Unchanged maximal renal concentrating capacity.

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The Effects of Cimetidine (Tagamet®) on Renal Function in Patients with Renal Failure

Rutger Larsson Goran Bodemar Bertil Kågedal and Anders Walan

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ABSTRACT Cimetidine has been administered during 7 days to 28 patients with different degrees of renal failure. Thirteen of these patients had a further week's treatment at least one month later. The daily dose of cimetidine was reduced in relation to pretrial values of creatinine clearance. There was a clear rise in serum creatinine all through the trial ($22.3 \pm 2.6\%$ at day 7) ($p < 0.001$). Maximal decreases in creatinine clearance occurred on day 2 ($21.8 \pm 2.2\%$) and day 3 ($23.2 \pm 0.9\%$) ($p < 0.001$), but were still present on days 6 ($16.4 \pm 2.9\%$) and 7 ($17.3 \pm 2.8\%$) ($p < 0.001$). There was a small rise in serum uric acid all through the trial ($p < 0.05$). Three days after finished treatment there were no significant differences in serum creatinine, creatinine clearance and serum uric acid when compared to pretrial values. The pattern of changes in serum creatinine and creatinine clearance was the same in both mild and severe renal failure. Glomerular filtration rate determined by [^{51}Cr] EDTA clearance before, on day 3 of treatment and 3 days after treatment did not show any differences. No change was seen in serum β_2 -microglobulin during the trial. The decrease in creatinine clearance during treatment with cimetidine is probably not caused by a reduction of glomerular filtration rate, but could be explained by competition by cimetidine for tubular secretion of creatinine. Treatment with cimetidine of patients with renal failure may invalidate measurements of serum creatinine and creatinine clearance as standard routine tests for glomerular filtration rate.

Key words: cimetidine renal function renal failure inhibited tubular secretion creatinine uric acid

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In patients with peptic ulcer disease and normal serum creatinine there is a small but significant increase in serum creatinine usually within normal limits during the first weeks of treatment with

cimetidine (3 4 16 20). Values return to pretreatment levels during continued long term treatment (4). A decrease in creatinine clearance has been reported already after single oral or i.v. doses of cimetidine in healthy man but inulin clearance remained constant (10 14) and creatinine clearance normalized at the end of one week's treatment in high doses (10). In patients with peptic ulcer disease and normal renal function both creatinine and [^{51}Cr]EDTA clearances were decreased after one day of treatment with cimetidine 1.6 g/d (15). These decreases were also accompanied by a decrease in effective renal plasma flow determined with [^{125}I]hippuran clearance (15). After 3 and at the end of 12 weeks' treatment with cimetidine in the same doses the clearance values for creatinine [^{51}Cr]EDTA and [^{125}I]hippuran had returned to the pretreatment values (15). We have previously reported significant increases within normal limits in serum creatinine and serum β_2 -microglobulin after 2 and 6 weeks' treatment with cimetidine 1.0 g/d in patients with peptic ulcer disease and normal serum creatinine (20). However [^{51}Cr]EDTA and creatinine clearances were unchanged during treatment compared to pretreatment values (20). Cimetidine has been shown to be effective in the prophylactic treatment of upper gastrointestinal haemorrhage in renal transplant recipients (18) and could be of value in patients on regular haemodialysis (12 17). Nothing is known however about the effect of cimetidine on renal function in patients with established renal failure.

The aim of this study was to investigate the effect on renal function in patients with renal failure during treatment with cimetidine for one week. The doses were adjusted to the degree of renal failure according to the results of a previous oral single dose study (21).

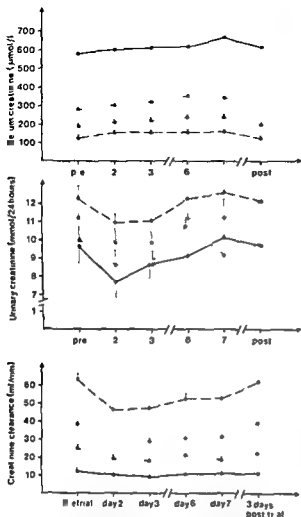


Fig 1 Serum creatinine 24-hour urinary creatinine and creatinine clearance (mean \pm SE) before during and after cimetidine treatment for 7 days in patients with a creatinine clearance of 5–15 ml/min (—) (5 treatment periods) 15–30 ml/min (---) (10 treatment periods) 30–50 ml/min (···) (13 treatment periods) and 50–75 ml/min (- · -) (13 treatment periods) * $p < 0.05$ ** $p < 0.01$ compared to pretrial values (Wilcoxon's test for paired differences)

PATIENTS AND METHODS

Twenty-eight outpatients (8 women and 20 men) mean age 56 years (range 29–77) with different degrees of renal failure were treated with cimetidine for 7 days. In 13 patients the investigation was repeated at least one month after the first investigation making a total of 41 treatment periods. Nine patients had glomerulonephritis, 9 nephroarteriosclerosis, 3 pyelonephritis, 3 interstitial nephropathy, 3 polycystic kidney disease and 1 patient diabetic nephropathy. They did not have apparent gastrointestinal disease and the renal function had been stable for a minimum of one month before the trial. All patients received their usual drug treatment during the trial including antihypertensives, diuretics, chemotherapeutics, al-

lopurinol and antacids. Even irregular intake of antiacids before and during the trial was recorded. All gave informed consent before entering the trial.

The doses of cimetidine were adjusted in relation to creatinine clearance values determined within a week before trial as follows: Cimetidine 200 mg 3 times daily given to 5 patients with a creatinine clearance of ml/min (5 treatment periods), 200 mg 3 times daily patients with a creatinine clearance of 15–30 ml/min (treatment periods), 200 mg 4 times daily to 8 patients a creatinine clearance of 30–50 ml/min (13 treatment periods) and 200 mg 3 times daily and 400 mg at bedtime 9 patients with a creatinine clearance of 50–75 ml/min (treatment periods).

Creatinine, urea, uric acid, phosphate, calcium, β_2 -microglobulin in serum and endogenous 24-hour creatinine clearance were estimated in duplicate 2 days before entry to the trial, on days 2, 6 and 7 during treatment and 3 days after treatment. In 15 patients with moderate degree of renal failure [^{51}Cr]EDTA clearance with a simplified single injection technique (5) was estimated 2 days before, on day 3 during and 3 days after treatment. Blood samples were drawn at 180, 200, 220, 240 min after i.v. injection of 100 μCi [^{51}Cr]EDTA. The clearance values of creatinine and [^{51}Cr]EDTA corrected to 1.73 m^2 BSA. Serum β_2 -microglobulin analyzed by a radioimmunosorbent technique (Phac β_2 -microglobulin test, Pharmacia Diagnostics Uppsala). Besides these laboratory investigations, routine blood and urine chemical analyses were performed during the trial.

The statistical analyses were performed with Student's paired t -test and Wilcoxon's test for paired differences. The results are given as mean \pm SE.

RESULTS

The effect of cimetidine on serum and urinary creatinine and creatinine clearance in the 4 groups of patients with renal failure during cimetidine treatment are given in Fig. 1. Mean 24-hour urinary excretion of creatinine decreased significantly on days 2 and 3 except in patients with a creatinine clearance of 5–15 ml/min. During continued treatment urinary creatinine excretion returned to pre-trial levels on days 6 and 7. There were statistically significant increases in serum creatinine and decreases in creatinine clearance in all groups of patients with renal failure except again for the group with a creatinine clearance of 5–15 ml/min. All values returned to the pre-trial values 3 days post-treatment. The mean percentage increase in serum creatinine for all 41 treatment periods was more pronounced on days 6 (21.8%) and 7 (22.3%) than on days 2 (13.8%) and 3 (16.3%). The mean percentage decrease in creatinine clearance seemed to be more pronounced during days 2 (21.8%) and 3 (23.9%) than during days 6 (16.4%) and 7 (17.3%).

Table 1 Urinary acid excretion and plasma urea nitrogen in 22 patients with renal failure before and after 33 treatment periods with cimetidine for 7 days (mean \pm S.E. range in parentheses)

Time relation to treatment	Serum uric acid (μ mol/l)	Serum calcium (mmol/l)	Serum phosphate (mmol/l)
Before treatment	4.5 \pm 17 (2.53–6.44)	2.46 \pm 0.01 (2.29–2.68)	1.13 \pm 0.03 (0.70–1.71)
During treatment			
Day 2	4.37 \pm 17	2.43 \pm 0.01	1.20 \pm 0.03
Day 3	4.38 \pm 16	2.40 \pm 0.01**	1.19 \pm 0.04
Day 6	4.46 \pm 17**	2.41 \pm 0.01**	1.10 \pm 0.04
Day 7	4.47 \pm 18	2.41 \pm 0.01**	1.16 \pm 0.04
3 days after treatment	4.5 \pm 17	2.40 \pm 0.01**	1.14 \pm 0.04

$p < 0.05$ ** $p < 0.01$ * $p < 0.001$ compared to results before treatment (Student's paired *t* test)

The 15 patients with a moderate degree of renal failure in whom [Cr]EDTA clearance was measured had a mean pretrial creatinine clearance of 11.3 ml/min and a mean [Cr]EDTA clearance of 17.3 ml/min (Fig. 2). Mean creatinine clearance decreased significantly during treatment and returned to the pretrial value 3 days after treatment. Mean [Cr]EDTA clearance on day 3 during treatment and 3 days after treatment was unchanged. Mean serum β_2 microglobulin concentration was unchanged during the trial (Fig. 2).

Uric acid, calcium and phosphate in serum were analyzed during 33 treatment periods in 27 patients (Table 1). The mean value of serum uric acid before

treatment was 475 μ mol/l and there were small but statistically significant increases of 2.8–6.1% from day 2 to day 7 with a return to the pretrial mean value 3 days posttrial. The accepted reference interval for serum uric acid is 140–340 μ mol/l for women and 200–470 μ mol/l for men. Mean serum calcium before treatment was 2.46 mmol/l (accepted reference interval 2.25–2.75) and decreased slightly but significantly from day 3 throughout the trial. Three days after treatment mean serum calcium had still not returned to the pretrial value. Mean serum phosphate before the trial was 1.13 mmol/l (accepted reference interval 0.6–1.6). There was a slight but statistically significant increase on day 2 during treatment with a return to the pretrial value during continued treatment. In 19 treatment periods in 16 patients not treated with antacids there was also a statistically significant increase in serum phosphate on day 3 during treatment ($p < 0.01$) as well as on day 7 ($p < 0.05$).

There were no significant changes in serum urea, transaminases, creatine phosphokinase and routine blood or urine tests during the trial. The patients had no side effects during the trial.

DISCUSSION

In our patients with different degrees of renal failure treated with cimetidine for one week, mean urinary excretion of creatinine decreased significantly on days 2 and 3 but returned to the pretrial value on days 6 and 7 during continued treatment. This caused significant increases in serum creatinine and decreases in creatinine clearance from day 2 to day 7 during treatment. The decreases in creatinine clearance seemed to be more pronounced on days 2

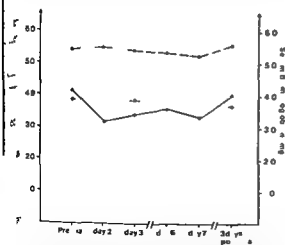


Fig. 2 Serum β_2 microglobulin (—) 24-hour creatinine clearance (---) and [Cr]EDTA clearance (---) during and after treatment with cimetidine for 7 days (mean \pm S.E.) in 15 patients with renal failure. $p < 0.05$ * $p < 0.01$ compared to pretrial values (Wilcoxon's test for paired differences).

and 3 compared to days 6 and 7 despite higher values of serum creatinine on days 6 and 7. This pattern may be interpreted in the following way: Reduction of urinary creatinine excretion causes accumulation of creatinine until a new steady state in which the daily quantity of excreted creatinine matches the amount retained by metabolism in each day is achieved on days 6 and 7.

The pattern and degree of changes in serum creatinine were the same in mild, moderate and severe renal failure. This resulted in more pronounced increases in serum creatinine in patients with severe renal failure. Mean serum creatinine in patients with a creatinine clearance of 5–15 ml/min before treatment was $512 \mu\text{mol/l}$ and on day 7 $671 \mu\text{mol/l}$, while in patients with a creatinine clearance of 50–75 ml/min the corresponding means were 126 and $155 \mu\text{mol/l}$ respectively (Fig. 1).

The rise of serum creatinine during cimetidine treatment could not be explained by an excessive increase in endogenous production of creatinine since creatine phosphokinase in this and other trials (10–20) did not change. This is supported by the fact that there was no increase in the urinary excretion of creatinine. The increase in serum creatinine could not be attributed to interference of cimetidine or its sulphoxide metabolite on analysis of creatinine in serum (19).

[^{51}Cr]-EDTA clearance, which is a fairly specific method for the determination of glomerular filtration rate and has an excellent correlation to creatinine clearance (6), was unchanged during this trial. Thus the changes in serum creatinine and creatinine clearance could not be explained by a reduction of glomerular filtration rate. The unchanged mean serum β -microglobulin during this trial supports the opinion that glomerular filtration rate is not influenced by cimetidine therapy in patients with renal failure.

Cimetidine is reported to be rapidly excreted via the kidneys mainly as unchanged drug (9). The mean renal clearance of cimetidine in normal man is 40–50 ml/min (19–23) which means that about 2/3 is secreted in the tubules. Creatinine is mainly cleared by glomerular filtration but small quantities are also secreted and possibly also reabsorbed in the tubules (1–34–25). The tubular maximum for secretion of creatinine exceeds that of reabsorption which explains why the creatinine clearance values in normal man are 5–10% higher than glomerular filtration rate determined by creatinine clearance (13).

In renal failure creatinine clearance may exceed glomerular clearance by as much as 50–100% (13). The creatinine clearance/[^{51}Cr]-EDTA clearance ratio before treatment in our patients was 1.11 and increased by day 3 to 0.89. This might be explained by inhibited tubular secretion of creatinine caused by competition by cimetidine for the same tubular transport system. Reabsorption of creatinine therefore dominates over secretion.

Tubular secretion by active transport is effected through an acid or base secreting system (1). Weak acids such as caronamide (4 carboxy-phenyl-methanesulphonyl amide) (18) and salicylate (11) are known to decrease the tubular secretion of creatinine in man, thus proving that creatinine is secreted by the secretory system for weak acids. Berglund et al. (2) however reported that trimethoprim, which like cimetidine is a weak base, also inhibits the tubular secretion of creatinine. They concluded that creatinine secretion in man also might take place through the base transport system as well.

Mean serum urea and in our patients was slightly above the accepted reference interval before treatment, as a result of renal failure and/or diuretic therapy. There was a small but significant increase in mean serum urea and during treatment, with return to the pretreatment value after withdrawal of treatment. Like creatinine urea and follows a proximal secretion pattern, i.e. secretion takes place at a site proximal to reabsorption (1). Unlike creatinine the tubular maximum for reabsorption exceeds that of secretion (1). Unconscious drugs like sodium salicylate and probenecid increase the renal excretion of urea and due to inhibited tubular reabsorption (22). However in small doses these drugs have been reported to decrease the excretion of urea and due to inhibition selectively of the tubular secretion without effect on tubular reabsorption (24). It thus seems possible that cimetidine like salicylate could compete with urea and as well as creatinine for secretion in the tubules.

The slight changes in calcium and phosphate in serum observed in this study are difficult to explain but are probably not due to any primary effect of cimetidine on renal function.

In summary treatment with cimetidine does not seem to cause any impairment of glomerular filtration rate in patients with renal failure. The observed increase in serum creatinine and decrease in creatinine clearance might be caused by inhibited

tubular secretion of creatinine which is reversed after withdrawal of treatment. This may however be of clinical importance since cimetidine treatment may invalidate serum creatinine and creatinine clearance as standard routine tests of glomerular filtration rate. This is especially important in renal transplant recipients during treatment with cimetidine in whom a rise of 15–20% in serum creatinine would make a transplant rejection suspect and might lead to an increase in immunosuppressive therapy. Serum β microglobulin should perhaps be used as a complementary simple renal function test to monitor renal transplant function in patients treated with cimetidine. Further renal function investigations are needed in patients with renal failure and peptic ulcer disease treated with cimetidine to evaluate whether the changes in serum creatinine and creatinine clearance are reversible during longer treatment periods as has been described in patients with normal renal function (4).

ACKNOWLEDGEMENTS

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Serum and Urinary Myoglobin in Alcoholics

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ABSTRACT The serum levels of myoglobin were measured in 106 male alcoholics. Subnormal levels were found in 31% of the alcoholics with no alcohol consumption for the last 2-4 weeks while none of them had elevated levels. Of the alcoholics on ambulatory control and with varying current alcohol intake, 18% had increased levels compared to healthy controls. Serial myoglobin levels were determined in 19 patients following the cessation of heavy drinking sprees. Despite the fact that none of the patients had clinical evidence of acute myopathy, marked myoglobin elevations were noted in five patients; their serum levels gradually declined and normalized within 4-7 days. Comparing the three groups, similar frequencies of subnormal or elevated serum CK levels were observed. Myoglobin levels were not raised due to impaired glomerular filtration rates. No correlation was found between serum myoglobin and laboratory signs of liver affection. Although hypophosphatemia, hypomagnesemia and hypokalemia were occasionally noted, decreased serum electrolytes did not have any relation to elevated serum myoglobin. A transient, slightly increased urinary excretion of myoglobin parallel with increased excretion of β -microglobulin was observed in 2/17 alcoholics, suggesting that instances of myoglobinuria in alcoholics with heavy recent drinking may be due to a transient minor tubular dysfunction.

Key words: alcoholics, serum myoglobin, urinary myoglobin, liver, kidney.

Acta Med Scand 208 33-39 1980

The circulating levels of the low molecular weight heme protein myoglobin have been reported to be elevated in conditions with damage to striated or myocardial muscles (5-9, 23). The elimination of myoglobin from the circulation occurs mainly by the renal route (1, 6) but evidently to some extent also by the liver (1, 21). Hence, elevated serum myoglobin is observed in patients with renal failure

(6) and slightly elevated in patients with hepatic failure (21). In alcoholics, both acute and/or chronic myopathies are observed (8) and accompanied by elevated creatine kinase (CK) levels (14). We report here the frequency of pathological myoglobinemia in alcoholics and the changes in serum myoglobin after a period of excessive drinking, possibly involving a disturbed elimination rate for this protein.

It has been argued that disturbance of the electrolyte balance is a mechanism underlying the myopathies of alcoholics (12, 15, 20). An attempt was therefore made to correlate elevated myoglobin levels to serum concentrations of various electrolytes. Since myoglobinuria has previously been reported in single cases with heavy alcohol consumption (7, 25), we also investigated the urinary excretion of myoglobin after alcohol abuse. The urinary excretion of β -microglobulin, another low mol wt protein normally almost completely reabsorbed by the proximal tubules (19), was simultaneously measured in order to elucidate whether myoglobinuria in alcoholics might be due to tubular dysfunction.

PATIENTS AND METHODS

This study comprised three groups of male alcoholics. *Group A* consisted of 42 non-selected chronic alcoholics (mean age 43, range 24-67 years) who, due to excessive drinking, were admitted involuntarily for institutional care at Västman County of Stockholm, Sweden. They had histories of alcoholism of 7-20 years duration. Serum samples were collected from these patients 2-4 weeks after admission, during which time they had been prevented from taking alcohol. *Group B* consisted of 19 patients with histories of alcoholism of 5-20 years admitted to the Department of Psychiatry, Uppsala, after a

Abbreviations: CK = creatine kinase, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase, γ GT = gamma glutamyl transferase, GFR = glomerular filtration rate.

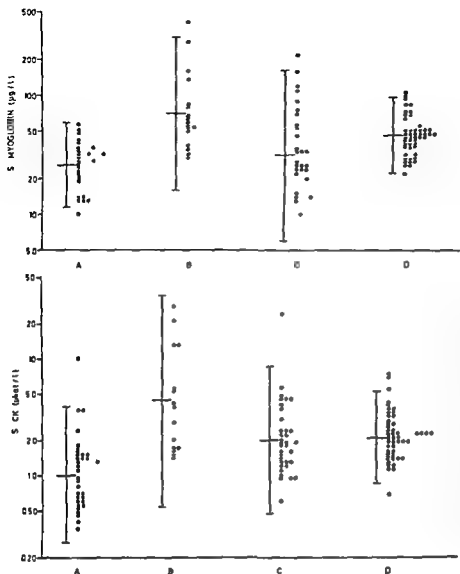


Fig 1 Mean levels of myoglobin and CK in sera of male alcoholics (A, B) and healthy age-matched men (D). Geometric means of myoglobin (ranges expressed as 95% confidence limits) were for group A 26.0 (11.5–58.7) $\mu\text{g/l}$, group B 71.2 (16.2–312.3) $\mu\text{g/l}$, group C 32.5 (6.4–165.3) $\mu\text{g/l}$, group D 47.3 (22.6–98.1) $\mu\text{g/l}$. Corresponding means (ranges expressed as 95% confidence limits) for CK were for group A 10.2 (2.7–39.3) $\mu\text{kat/l}$, group B 44.0 (10.5–35.36) $\mu\text{kat/l}$, group C 20.2 (4.7–2.49) $\mu\text{kat/l}$, group D 21.0 (8.2–54.4) $\mu\text{kat/l}$.

period of alcohol abuse of 3–16 weeks. Individuals in this group had on average consumed 340 g alcohol/day (range 180–480) during the week prior to admission and were monitored by serial determinations during 14–16 days from admission to hospital. They were treated with chlormethiazole during the first week and disulfiram daily during the observation period. Two patients developed delirium tremens at the beginning of the study. Sera were collected at least three times and from the majority of patients on eight occasions during the observation period. Urine was collected during 24 hours on days 1, 7 and 11. Group C: Serum samples were collected on one occasion from 45 consecutive outpatients (mean age 41, range 21–62 years) with alcoholism of varying duration (5–20 years) ambulatory-controlled as part of a voluntary weaning program at the Department of Psychiatry, University Hospital Uppsala, Sweden. The degree of alcohol consumption during the period nearest blood collection probably varied within wide limits and approximately 20% of the patients were treated with disulfiram.

All sera were subjected to the following analyses (reference ranges are given in parentheses with 95% confidence limits): Alanine aminotransferase (ALAT) ($<0.6 \mu\text{kat/l}$) and aspartate aminotransferase (ASAT) ($<0.6 \mu\text{kat/l}$) according to the methods recommended by the Commission on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (22). The coefficients of variation of the methods were 1.5 and 2% respectively. Bilirubin ($0.8\text{--}4.8 \mu\text{kat/l}$) according to Michaelsson with a coefficient of variation of 2%. CK and γ -glutamyl transferase (γ -GT) ($<1.0 \mu\text{kat/l}$ for males) were measured with commercially available kits and according to manufacturer's instructions (Boehringer Mannheim, Germany). The coefficients of variation for the methods were 4 and 3% respectively. Creatinine ($64\text{--}106 \mu\text{mol/l}$) was measured by an autoanalyser technique (Technicon 11b). The coefficient of variation for the method was approximately 2%. Myoglobin was measured as previously described (21) by a radioimmunosorbent assay. The coefficient of variation for the assay was 7%.

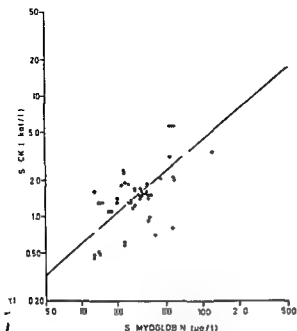


Fig 2 Serum levels of myoglobin and CK in the 106 alcoholics illustrated in Fig 1. The equation for the regression line is $\log \text{CK} = 0.861 \log \text{myoglobin} - 1.086$, coefficient of correlation 0.79 ($p < 0.001$).

The sera obtained from the longitudinally studied 19 patients were also analysed for the following electrolytes: calcium (1.44–2.20 mmol/l), potassium (3.2–5.1 mmol/l), magnesium (0.65–1.00 mmol/l) and phosphate (0.76–1.44 mmol/l). Calcium and magnesium were analysed by an atomic absorption spectrophotometric method, potassium by flame photometry and phosphate by an autoanalyser technique (Technicon). The coefficients of variation of the methods were 1.5, 2, <1.0 and 2% respectively.

An iv galactose tolerance test was performed on the 19 patients on the 8th or 10th day after admission. The test, which was performed according to Tengström (24) using 1.94 mmol/galactose/kg body mass, has in our laboratory an upper $T_{1/2}$ limit of 17 min for a normal adult.

Urine specimens were collected in plastic bags of 2 l capacity with two ml of a preservative solution (2 mol/l Tris HCL buffer, pH 8 with 0.2% sodium azide) and analysed for β_2 -microglobulin and myoglobin (21). The normal excretion of β_2 -microglobulin is 30–370 $\mu\text{g}/24$ hours and for myoglobin <5 $\mu\text{g}/24$ hours.

Student's t test was used for statistical analyses on groups or paired values.

RESULTS

Serum and urinary myoglobin concentrations

Fig 1 shows serum myoglobin concentrations in 57 healthy male controls and 106 male alcoholics. The

latter divided into three groups. 18% of the ambulatory-controlled patients (group C) had myoglobin values above the reference range. None of the patients who had not consumed alcohol for 2–4 weeks (group A) had elevated serum myoglobin levels. As a group they had significantly reduced serum myoglobin concentrations compared with the controls ($p < 0.001$) and 31% of the patients had subnormal values. Group II, which comprised 19 alcoholics admitted to the hospital due to excessive drinking in recent weeks, was followed by serial serum determinations from the day after admission. At the time of the first serum sample, five of the patients had elevated myoglobin levels, while the others had levels within the control range. Serial determinations showed a uniform pattern of declining myoglobin values during the first week after admission, with a few exceptions. An initial decline was followed by a sharp rise in serum myoglobin in two patients. All elevated serum levels normalized within the observation period of 2 weeks.

A significant correlation ($r = 0.79$, $p < 0.001$) was found between serum CK and serum myoglobin concentrations (Fig 2) and the frequency of serum CK outside the control range was quite similar among the alcoholics (for the whole material as well as for the three groups) to that of myoglobin (Fig 1). Serial determinations of serum CK also revealed the same pattern of changes as for serum myoglobin (Fig 3). Two patients developed delirium tremens on the second day after admission but no rise in serum myoglobin or CK was observed in connection with these attacks.

Only two patients had urinary excretion of myoglobin above the upper normal limit on the first day. These two patients also had increased urinary excretion of β_2 -microglobulin (>370 μg). The urinary excretion of both proteins returned to normal during the observation period (Fig 4). None of the patients had clinical signs of muscle affection such as severe muscle tenderness, edema or apparent weakness.

Relation of kidney and liver function to serum myoglobin

No patient in the entire material had an impaired glomerular filtration rate (GFR) as judged from serum creatinine, nor were any significant changes observed in this variable in the patients who were followed by serial determinations, indicating that

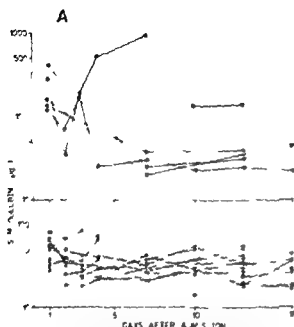
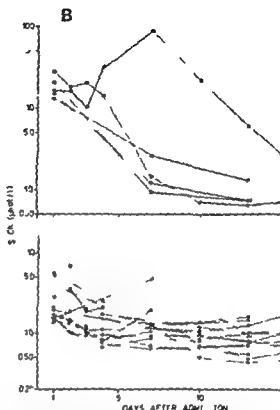


Fig. 1 (A) Serial determinations of myoglobin in 19 alcoholics admitted to hospital after a period of excessive drinking divided into two subgroups on the basis of serum



myoglobin on admission: one with elevated serum myoglobin ($>98.8 \mu\text{g/l}$) and the other with serum myoglobin within the control range. (B) Serial determinations of

elevated serum myoglobin could not be attributed to an impaired GFR.

Another cause of disturbed myoglobin elimination could be reduced liver function. The mean serum concentrations of liver enzymes and bilirubin in the three groups of alcoholics are listed in Table 1. Although signs of liver affection were frequent findings, there was no correlation between the serum levels of myoglobin and bilirubin ($r=0.06$), ASAT ($r=0.14$) or γ -GT ($r=0.22$). Six of the 19 longitudinally followed in patients had impaired lactose elimination rates but no correlation was found between this estimate of liver function and serum myoglobin concentrations on days 1 or 10 ($r=0.34$ and 0.24 respectively).

Relation of serum electrolytes to serum myoglobin

On admission we observed in a few of the serially followed patients slightly reduced (within the minus 2 to 3 S.D. intervals) serum concentrations of phosphate (4 cases), potassium (3 cases) and magnesium (3 cases). By the fourth day the concentrations had

normalized. Significant increments ($p<0.05$) in relation to the first value were found for phosphate days 4 and 7 and for potassium on day 13 (Table 1). However, no correlation was found between myoglobin and electrolyte levels. Nor did analysis of the individual values give rise to the suspicion of any connection between changes in serum myoglobin and electrolytes.

DISCUSSION

The analysis of serum concentrations of myoglobin in male alcoholics shows that the circulating level of this protein are elevated in a considerable number of patients without clinical myopathy. Muscle affections in alcoholism are rare entities; the clinically recognizable forms of acute and chronic alcoholic myopathy (8). However, subclinical myopathy as detected by estimation of CK is reported in 38–84% of chronic alcoholics (13, 17, 26). In this study a comparison of serum level of myoglobin and CK showed excellent agreement

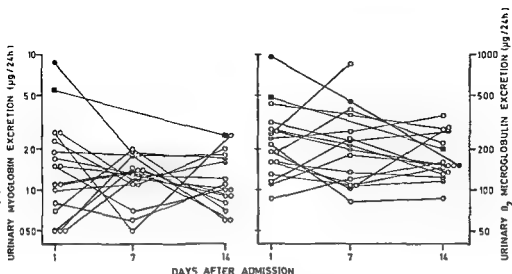


Fig 4 Urinary excretion of myoglobin and β_2 microglobulin during a 2 week abstinence period in 17 alcoholics admitted to hospital after heavy drinking. \bullet \blacksquare = Patients

with increased urinary excretion of myoglobin ($>5 \mu\text{g}/24 \text{ hours}$). The upper limit of normal excretion for β_2 microglobulin is $370 \mu\text{g}/24 \text{ hours}$.

suggesting that previous reports on CK values in alcoholics are comparable with present data on myoglobin. Taken as a whole 10% of the alcoholics in this study had elevated myoglobin levels. However when the patients were divided into three groups according to the probability and degree of current alcohol consumption or abstinence from alcohol quite different frequencies were obtained. In the group of chronic alcoholics who had received institutional care and been without alcohol intake for 2-4 weeks nobody had elevated serum myoglobin or CK, while about one third had subnormal serum concentrations. In the group of chronic alcoholics with heavy alcohol consumption until admission 26% had pathologically raised serum myoglobin concentrations. Of the alcoholics under ambulatory control in an alcohol weaning program and with vague or uncertain information as to the true alcohol intake 18% had elevated serum

myoglobin values. Serial determinations of myoglobin and CK in alcoholics after alcohol abuse showed their steady parallel decrease and final normalization 4-7 days after admission and with withdrawal of alcohol.

Myoglobin is mainly eliminated by the renal route (6) but animal experimental studies (1) and our previous observations (21) indicate that myoglobin also to a minor extent is eliminated by the liver. The possibility that impaired renal function influenced our results can be ruled out. Although many of the patients had laboratory signs of impaired liver function we found no correlation between the myoglobin levels and the degree of liver affection. Taken together, this suggests that elevated myoglobin is caused mainly by increased myoglobin release from muscles indicating a muscular affection which in view of the rapid normalization of serum myoglobin appears to be quite temporary after withdrawal of

Table 1 Laboratory liver findings

The values are given as arithmetic means \pm SD

	Bilirubin ($\mu\text{mol/l}$)	ASAT ($\mu\text{kat/l}$)	ALAT ($\mu\text{kat/l}$)	γ GT ($\mu\text{kat/l}$)	$\text{T} \frac{1}{2}$ galactose (min)
Group A	9.09 ± 7.25	1.12 ± 0.94	1.25 ± 1.14	2.94 ± 2.75	—
Group B	7.19 ± 5.30	0.79 ± 0.67	0.83 ± 0.66	2.33 ± 2.80	
Group C	12.55 ± 4.27	1.56 ± 1.13	1.21 ± 0.80	2.50 ± 2.26	

Table II Serum electrolytes in alcoholics serially followed from start of abstinence (mean \pm S D)

	Day			
	1	4	7	13
Phosphate	0.95 \pm 0.21	1.10 \pm 0.31	1.16 \pm 0.24	1.08 \pm 0.25
Calcium	2.42 \pm 0.17	2.30 \pm 0.17	2.44 \pm 0.16	2.46 \pm 0.11
Potassium	3.74 \pm 0.64	3.95 \pm 0.41	4.03 \pm 0.48	4.28 \pm 0.47*
Magnesium	0.75 \pm 0.15	0.77 \pm 0.09	0.94 \pm 0.10	0.80 \pm 0.10

* Significant increment ($p < 0.05$) compared with day 1 and calculated on paired values

alcohol. In contrast the subnormal myoglobin levels found in alcoholics may persist at least a month with total abstinence possibly reflecting a reduced muscle mass due to malnutrition or alternatively a more chronic atrophic muscular disorder induced by alcohol. The reports on clinically overt myopathies also indicate that patients with acute alcoholic myopathy recover rapidly and evidently completely but that patients with long lasting alcoholic myopathy have muscular disability for longer periods after discontinued drinking (4).

The pathogenesis of the muscular affection in alcoholics is not yet clear. Among the hypotheses suggested one could mention prolonged ischemia (3), nutritional deficiency (2), depression of glycolytic enzymes (18) and destruction of the muscle cell membranes and mitochondria (11). It has also been suggested that there is a primary disturbance of water and electrolyte metabolism in chronic alcoholics with secondary development of intracellular edema and cell damage. Since hypokalemia, hypomagnesemia and hypophosphatemia are known to occur in alcoholism (12-15, 20) and all are known to be associated with changes in muscle function and/or histology (4, 20) we searched for a relationship between the serum levels of these electrolytes and myoglobin. We observed hypophosphatemia as well as hypomagnesemia and hypokalemia in certain alcoholics on admission and also significant increments with time in the concentrations of phosphate and potassium in the entire patient group. However we found no correlation between myoglobin levels and serum electrolytes. Deficiency of these electrolytes may contribute to the muscular affection but it does not seem to be the primary cause of elevated serum myoglobin or CK in alcoholics.

Individual cases with the most dramatic form of acute alcoholic myopathy, alcoholic rhabdomyolysis, are reported to have myoglobinuria

and also acute renal failure (7, 25). The development of a radioimmunoassay for the measurement of low concentrations of myoglobin in urine (21) has enabled the study of urinary myoglobin excretion in alcoholics before the appearance of acute severe myopathy. We found that 2/17 cases had myoglobin excretion above the control range after re- drinking. However in these cases we also found increased excretion of β microglobulin—a mark of tubular function (19)—which normalized after four days of alcohol abstinence, as did the myoglobin excretion. These data are in accordance with previous observations indicating a fairly high renal threshold for myoglobin in normal situations but an increased excretion in situations with tubular dysfunction (10). Thus the present results suggest that a transient tubular affection may be seen in alcoholics and as a secondary phenomenon increased urinary output of myoglobin.

Whether the detection of elevated serum myoglobin or CK as a single sign of muscular affection in alcoholics is of clinical value is at present unsettled. Moreover there are some arguments against the estimation of serum myoglobin as a screening test for alcoholic myopathy. Muscular exercise may provoke a slight increase in serum myoglobin (21) and alcoholics usually have tremors and seizures during the withdrawal period. These conditions appear to be minor contaminants judging from the lack of a rise of myoglobin in two patients during attacks of delirium tremens. However many alcoholics are admitted to hospital due to trauma and since traumatic muscular damage certainly produces highly elevated myoglobin levels this is a factor to consider. In selected patients with clinical suspicion of severe acute or chronic alcoholic myopathy estimation of serum and urinary myoglobin may be of importance. At present it can clearly be stated that heavy drinkers with acute diffuse chest pains present a differential diagnosis

problem when serum myoglobin determination is used as an acute diagnostic test of myocardial infarction

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Serum Lipids with Special Reference to HDL Cholesterol and Triglycerides in Young Male Survivors of Acute Myocardial Infarction

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ABSTRACT: Serum cholesterol, triglycerides and high density lipoprotein (HDL) cholesterol were determined in 46 male survivors of myocardial infarction (MI) suffered 6-20 months earlier and in 82 healthy age- and sex-matched controls. The mean age of both groups was 39 years. Total serum cholesterol was within normal limits in both groups, whereas HDL cholesterol was considerably lower and triglycerides remarkably elevated in MI patients compared with the controls. Judging from previous and present data, low HDL cholesterol seems to be a coronary risk factor as such. This study indicates that low HDL cholesterol and especially a low HDL cholesterol/total cholesterol ratio may be stronger coronary risk factors than total serum cholesterol alone. Because HDL cholesterol and triglycerides were inversely correlated, these results also emphasize the importance of serum triglycerides as a risk factor for coronary heart disease.

Keywords: Cholesterol, triglycerides, high-density lipoprotein, myocardial infarction.
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During the last five years plasma high density lipoprotein (HDL) cholesterol has received increasing attention as an independent risk factor for coronary heart disease (CHD) (10, 12, 15). In the early 50s, low α lipoprotein levels (i.e. HDL) were found in association with CHD, even in young males (13, 14). Unfortunately, triglycerides were not commonly measured at that time. Later work on the role of lipids in CHD mainly focused on plasma total cholesterol. Elevated triglycerides have only occasionally been associated with an increased occurrence of CHD.

Since triglycerides and HDL cholesterol have been found to be inversely related (17), the present

work was undertaken to elucidate the role of total cholesterol, HDL cholesterol and triglycerides in young male survivors of acute myocardial infarction (MI).

PATIENTS AND METHODS

Fifty-six male subjects (mean age 39 years, range 24-45) were studied 6-20 months after definite MI. They had been treated in the Departments of Medicine, University Central Hospital of Helsinki, and diagnoses were established from clinical histories, serial ECGs and blood analyses fulfilling the WHO criteria (19). This was the first infarction in these patients and none had diabetes mellitus. During hospitalization, routine dietary instructions were given to all subjects. At the time of the present investigation, 34 patients reported that they had significantly reduced the use of saturated fats and/or carbohydrates after MI. β -Blocking drugs were used by 39 patients (propranolol 7, metoprolol 7, alprenolol 3). Diuretics were given to 8 patients.

The control group consisted of 82 healthy male subjects with various occupations similar to those of the MI patients. The mean age was 39 years (range 29-40).

Venous blood was drawn for blood analyses after an overnight fast. Serum total cholesterol (1), triglycerides (9) and HDL cholesterol using precipitation by the PEG-6000 method (18) were determined. The division of hyperlipoproteinemia into subgroups was made according to the WHO recommendation (2). Serum total cholesterol and triglycerides were regarded as elevated if or above 7.0 and 1.7 mmol/l, respectively.

RESULTS

Serum total cholesterol and triglycerides, HDL cholesterol and the percentage HDL cholesterol of total cholesterol are given in Table I. Mean serum

Abbreviations: HDL, high-density lipoprotein; CHD, coronary heart disease; MI, myocardial infarction; LPL, lipoprotein lipase.

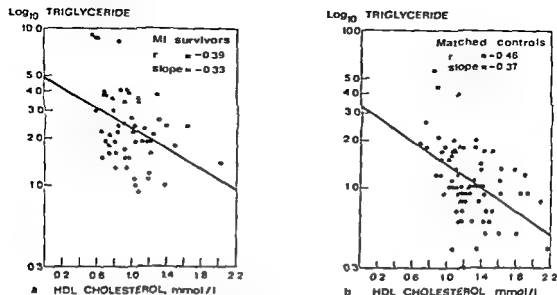


Fig. 1 Relationship between \log_{10} serum triglycerides and HDL cholesterol for MI survivors (a) and controls (b).

total cholesterol was within normal limits in MI patients though significantly higher than in controls. However, mean serum triglycerides were remarkably high and HDL cholesterol was very low in MI survivors compared to the controls. The HDL cholesterol levels of both MI survivors and matched controls were inversely correlated with the log of triglycerides ($r = -0.39$ and -0.46 respectively) (Fig. 1). The slope of the regression line was steeper in controls than in MI patients.

Thirty-nine patients (70%) were treated with β blocking drugs. Their mean HDL cholesterol (\pm S.D.) was 0.98 ± 0.21 mmol/l, which did not differ from the mean value of the non-users (0.92 ± 0.25 mmol/l).

DISCUSSION

Serum lipid and lipoprotein levels change during acute MI but return to their normal values within

2–3 months (7, 16). The time of entering the study is therefore important. In the present study lipids and lipoproteins were measured 6–20 after infarction, which is long enough not to date the results.

The present investigation indicated type hyperlipoproteinemia in only 7% of the MI survivors. This is a remarkably low incidence compared with the figure of 22% for an unselected Finnish population of the same age and sex (3).

The MI survivors comprised all male MI patients in this age group who were admitted to this hospital during the stated period, excluding those who in the acute phase it is improbable that the latter would have had type IIA hyperlipoproteinemia. Gustafson et al. (11) reported type hyperlipoproteinemia in 17% of MI survivors determined one year after MI.

Serum total cholesterol is considered to be

Table I Serum lipids and HDL cholesterol (mmol/l) mean \pm S.D. in MI survivors and controls

	n	HDL cholesterol	Total cholesterol	% HDL cholesterol	Triglycerides
MI survivors	56	0.98 ± 0.29 *	6.6 ± 1.2 *	15.2	2.6 ± 1.8
Normal lipids	13	1.11 ± 0.33	5.9 ± 0.6	18.8	1.3 ± 0.2
IIA	4	0.89 ± 0.15	7.6 ± 0.5	11.8	1.3 ± 0.3
IIB	20	0.93 ± 0.25	7.9 ± 0.8	11.9	3.2 ± 1.9
IV	19	0.97 ± 0.27	5.8 ± 0.6	16.9	3.3 ± 1.9
Controls	12	1.31 ± 0.30	5.4 ± 0.9	24.2	1.0 ± 0.4

* $p < 0.001$ for the difference between MI survivors and controls.

strong coronary risk factor especially in young men. Opinions on triglycerides have varied. Hypertriglyceridemia with or without associated hypercholesterolemia occurred in 70% of our MI survivors which is in agreement with earlier studies that have stressed serum triglycerides as a coronary risk factor (4, 5, 20).

Recently epidemiological evidence from studies in Hawaii, Framingham, Norway etc. have indicated an inverse relationship between HDL cholesterol and CHD (10, 12, 15). Our findings indicating markedly low HDL cholesterol values in young male survivors of MI compared with controls are in agreement with previous data derived from different populations. HDL cholesterol levels both in MI patients and controls were inversely related to triglyceride levels as noted previously. HDL cholesterol arises as a result of lipoprotein lipase (LPL) activity from very low density lipoprotein. The correlation between the activity of LPL and HDL cholesterol is positive but it is suggested that the ability of HDL to activate LPL is reduced in hypertriglyceridemic states (6). On the other hand it has been found that a reduction of serum triglycerides does not increase HDL cholesterol (8).

Our findings indicate that HDL cholesterol and the HDL cholesterol/total cholesterol ratio are stronger coronary risk factors than total cholesterol alone in young men with previous MI. More confirmation is however needed as to whether the reduction of HDL cholesterol really precedes the onset of clinical CHD.

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Effect of Guar Gum on Body Weight and Serum Lipids in Hypercholesterolemic Females

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ABSTRACT The effect of guar gum (15 g/day) on serum lipids and body weight of middle aged hypercholesterolemic females was studied in a double blind controlled trial. No consistent changes were observed in serum cholesterol, triglycerides or HDL cholesterol in patients taking guar gum, placebo or no medication at all. A highly significant decrease in body weight (62.9 ± 2.1 vs 60.4 ± 2.2 kg, $p < 0.0005$, paired comparison) was seen in subjects receiving guar gum, whereas body weight remained constant in the other two groups. It is concluded that the daily ingestion of 15 g of guar gum results in a permanent weight loss but does not influence serum lipids in females with hypercholesterolemia.

Keywords: guar gum, dietary fiber, weight loss, serum lipids.

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It has been suggested that low intake of dietary fiber is one of the factors responsible for the high prevalence of coronary heart disease in western society.

(1) The fiber hypothesis is partially supported by epidemiological studies indicating an association between the intake of fiber containing foodstuffs and the incidence of ischemic heart disease (8). Furthermore, there is some evidence that diet rich in fiber may protect against the risk factors of atherosclerosis like dyslipidemia, obesity and diabetes (11, 12, 13).

Despite numerous investigations it is not yet clear whether fiber added to the modern diet influences body weight or serum lipoprotein levels (7). The conflicting results are probably due to several factors. Firstly, it is becoming increasingly clear that the effects of various kinds of fibers are not identical. Secondly, the experimental design used in these investigations has not always been satisfactory in the absence of an adequate control group.

confounding factors like seasonal variation are likely to distort the results.

We report on the effects of guar gum, a viscous fiber containing galactose and mannose, on serum lipoproteins and body weight. The results of the randomized double blind study suggest that ingestion of moderate amounts of guar gum before meals induces weight loss but does not affect serum lipids in middle aged hypercholesterolemic females.

STUDY POPULATION AND METHODS

Subjects and design of the study

The subjects were recruited from a group of individuals with hypercholesterolemia detected in a screening operation aimed at primary prevention of coronary heart disease. Before entering the trial all subjects received diet instructions recommending a reduction of the use of saturated fats and simple carbohydrates and avoidance of excessive alcohol consumption. Weight loss was not encouraged. Antihypercholesterolemic medication, if there was any, was discontinued at least one month before the baseline measurements. Informed consent was obtained from all participants before the beginning of the investigation.

All subjects visited the municipal health center for blood lipid measurements three times at 4-week intervals before the trial. A total of 33 females with serum cholesterol levels above 7.6 mmol/l in all determinations were included in the study and were randomized into three experimental groups: guar gum (group A), placebo (group B) and no treatment (group C). One subject in group A did not complete the trial for an unspecified reason. Her values are not included in the calculations.

A double blind experimental design was used in the comparison of groups A and B. The subjects took either 5 g of guar gum or a placebo preparation (wheat flour containing no fiber) similar in appearance three times a day before meals. Guar gum was administered in granules 1 g containing 0.73 g of pure guar gum (Remeda Pharmaceutical Co., Kuopio, Finland). The patients were advised to mix the preparations with water.

Table 1 Serum cholesterol concentration (mmol/l) in groups A, B and C during the trial (mean \pm S.E.M.)

Group	Before treatment	Months after the beginning of treatment			
		1	2	3	4
A (n=10)	8.28 \pm 0.39	7.89 \pm 1.16	8.51 \pm 1.24	7.67 \pm 0.91	8.00 \pm 0.73
B (n=11)	9.40 \pm 0.26	8.63 \pm 0.38	8.43 \pm 0.56	8.83 \pm 0.62	9.04 \pm 0.41
C (n=11)	9.13 \pm 0.49	—	—	—	8.67 \pm 0.47

food or drink. The subjects in group C did not take any medication during the trial.

Techniques

Total serum cholesterol, body weight and blood pressure were measured three times at one month intervals before the trial (baseline measurements) and four times at monthly intervals during the trial. Serum triglycerides were determined twice in the baseline measurements and at the end of the 4-month study. High density lipoprotein (HDL) cholesterol was measured immediately before and at the end of the treatment period.

All blood measurements were carried out after an overnight fast. Cholesterol and triglyceride concentrations were determined by an Autoanalyser II apparatus (Technicon Instruments, Tarrytown, NY) using enzymatic assay (Boehringer Mannheim GmbH, Germany) (9, 14). HDL cholesterol was determined after precipitation of very low and low density lipoprotein with dextran sulphate-manganese according to Kostner (3).

Statistical methods

Mean and S.E.M. were calculated with a desk computer. Mean values were compared with Student's *t* test. Values expressed represent the mean \pm S.E.M. unless otherwise stated.

RESULTS

The mean concentrations of serum cholesterol, triglycerides and HDL cholesterol during the study are shown in Tables I and II. Serum cholesterol decreased slightly in all three experimental groups during the 4-month observation period, but the changes were not statistically significant. The con-

centration of HDL cholesterol remained constant in all groups. A statistically significant decrease in serum triglycerides was seen in the subjects receiving placebo, whereas no changes were seen in the other two groups.

The effect of fiber administration on body weight is shown in Fig. 1 and Table III. A consistent, highly significant decrease was seen in subjects treated with guar gum, whereas no change was observed in the other two groups. The decrease in body weight in group A was somewhat steeper in obese subjects (relative body weight $>115\%$) than in individuals with normal body weight, but the difference was not statistically significant (data not shown). No correlation was found between the change in body weight and serum lipids in any of the experimental groups.

Gastrointestinal symptoms reported by the participants in groups A and B during the study are shown in Table IV. Diarrhea, gastric pain, flatulence were more frequent among the subjects treated with guar gum than among those who received placebo, but the difference between the groups was not significant.

DISCUSSION

The results of this investigation indicate that addition of 15 g of guar gum to the Finnish diet did not produce permanent changes in serum lipids.

Table II Serum triglyceride and HDL cholesterol concentrations (mmol/l) in groups A, B and C at the beginning and end of the trial (mean \pm S.E.M.)

Group	Triglycerides		HDL cholesterol	
	Before	At 4 mo	Before	At 4 mo
A (n=10)	1.84 \pm 0.24	1.79 \pm 0.25	1.69 \pm 0.13	1.69 \pm 0.12
B (n=11)	2.61 \pm 0.39	1.86 \pm 0.23*	1.41 \pm 0.15	1.50 \pm 0.14
C (n=11)	1.75 \pm 0.20	1.76 \pm 0.17	1.63 \pm 0.12	1.76 \pm 0.20

* The difference between initial and final values is significant ($p < 0.05$, paired comparison).

Table III Body weight (kg) in groups A, B and C during the trial (mean \pm SEM)

Group	Before treatment	Months after the beginning of treatment			
		1	2	3	4
(n 10)	62.9 \pm 2.1	61.3 \pm 2.1	61.2 \pm 2.1	61.3 \pm 2.2	60.4 \pm 2.2*
(n 11)	66.1 \pm 4.0	65.4 \pm 3.3	65.9 \pm 3.7	66.3 \pm 4.0	65.7 \pm 3.7
(n 11)	63.3 \pm 2.9	—	—	—	61.7 \pm 2.9

The difference between initial and final values is statistically significant ($p < 0.0005$ paired comparison)

ipoproteins in female subjects with hypercholesterolemia. Lack of effect of guar gum on serum lipids is seemingly in disagreement with some earlier studies (5, 6). One explanation of the conflicting results might be the dose of guar gum. Thus the amount of fiber used by us (15 g/day) is distinctly lower than the dose reported to reduce serum cholesterol in healthy males (36 g/day) (5). Also in contrast to most of the previous investigations all subjects in our study had hypercholesterolemia (type A or II B abnormality) and there is evidence to suggest that the response of normal and hypercholesterolemic subjects to diet and various hypolipidemic agents is not identical.

The most intriguing finding of our study was however the highly significant decrease in body weight observed in the guar gum group. Indirect evidence for the weight reducing properties of a high fiber diet has earlier been obtained both from

epidemiological surveys and animal experiments (4). Moreover the few earlier studies conducted in human volunteers support the hypothesis that abundant intake of fiber prevents excessive food intake and induces loss of weight (2, 16). These observations are confirmed and extended by the results of the current double blind study: a highly significant weight reduction was seen in the guar gum group during the 4 month experiment where as no change was discernible in the two control groups.

The mechanism behind weight reduction by dietary fibers is not clear. In general diet rich in fiber has a lower calorie content and may also be less satiating than fiber depleted food. It has also been suggested that food fiber lowers calorie intake by promoting chewing and retarding the rate of food ingestion (4). These factors cannot however explain the present results since the subjects took the fiber with a small amount of food or drink before meals. Thus the most likely mechanism responsible for the weight loss is a decrease in the intestinal absorption of one or several components of the diet. In fact fecal excretion of fatty acids has been reported to increase during diets supplemented with fiber (10, 15). Similarly there is evidence that digestibility of protein is lowered by fiber (10). On the other hand despite a significant retardation the absorption of carbohydrates seems to be complete.

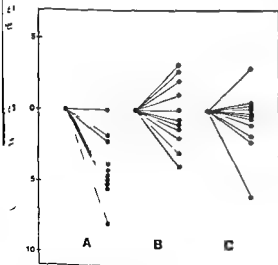


Fig. 1 Percentage change in body weight in the subjects of groups A (guar gum), B (placebo) and C (no medication) during the 4-month trial.

Table IV Frequency (%) of reported gastrointestinal symptoms (flatulence, gastric pain and diarrhea) in groups A and B during the trial

Group	Months after the beginning of treatment			
	1	2	3	4
A	80	60	50	60
B	36	72	36	45

even in the presence of large amounts of dietary fiber (6)

In summary, our results indicate that the addition of 15 g of guar gum to the Finnish diet induces a significant weight loss in middle aged hypercholesterolemic females over a period of at least four months. Despite the weight loss, no significant changes were observed in blood lipids or lipoproteins in this patient group under the conditions applied. It is suggested that regular ingestion of moderate amounts of fiber is a useful adjunct to the dietary therapy of obesity.

ACKNOWLEDGEMENT

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Hydralazine in Arterial Hypertension

Randomised Double Blind Comparison of Conventional/Slow Release Formulation and of b i d / q i d Dosage Regimens

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ABSTRACT Blood pressure (BP) control and tolerability of three two-week dosage regimens of hydralazine—conventional hydralazine q i d, conventional hydralazine b i d and slow release hydralazine b i d—were compared in a double blind randomized, cross-over trial in 20 out patients with arterial hypertension controlled with hydralazine in combination with other antihypertensive drugs. The efficacy of the treatments was assessed during the last 10 days of each treatment period by determination of BP and pulse rate every hour between 8 a.m. and 6 p.m. No statistically significant differences in BP and pulse rate were found between the three treatment regimens, either in the variation during the day or in the mean value for the day. There was a tendency to lower BP on conventional hydralazine q i d and to higher BP on conventional hydralazine b i d. Mean differences in supine BP between conventional hydralazine b i d and slow release hydralazine b i d were 3.2 systolic and 0.5 mmHg diastolic. In this short term study mean values with 95% confidence limits indicate that conventional hydralazine in a q i d dosage can be replaced by the same preparation in slow release formulation in a b i d dosage. A hypertensive phenotype was determined and had no significant influence on the results although there is a tendency to more widespread variability in systolic pressure and to a lower pulse rate in fast etylators. Unwanted effects were few and did not differ obviously between the treatments. Whether the frequency of late toxicity of hydralazine is lower with slow release formulation remains to be evaluated in long term studies.

Keywords: hydralazine, slow release, day curve of BP, etylator phenotype.

Med Scand 208 49 1980

or patient compliance with antihypertensive drug regimens is well documented (1 15 40). The pa-

tients usually without symptoms have to follow a long term treatment schedule which may involve polypharmacy and frequent dose regimens both of which foster poor compliance. Galley (11) and Malahy (25) found that the number of drug defaulters increased with the frequency of doses and according to Ayd (3) over 80% of patients who were to take four or more drugs at least three times a day failed to take 25–60% of the prescribed dose of each, whereas only 30% of those instructed to take one drug twice daily failed to take up to one fourth of the dose prescribed.

Consequently a one or two-dose schedule has been introduced for β blocking agents such as propranolol (2) pindolol (10 14 18) metoprolol (34) and atenolol (26 42). Recently slow release formulations of propranolol (6 27) and oxprenolol (8 19 30) have become available for administration once a day.

Hydralazine was introduced as a hypotensive agent in the early 50s. Following the realisation that late toxicity with LED syndrome is dose related hydralazine in doses up to 200 mg daily has proved a valuable and efficient antihypertensive drug in combination with other preparations. Since the introduction of β receptor blockers growing interest has been paid to the vasodilating effect of hydralazine as the combination with β -blockers prevents reflex tachycardia and hyperkinesia after hydralazine (22 36). It has been the practice to administer hydralazine in four doses per day as plasma half life is about three hours. A simpler dosage regimen would be of great interest.

The present investigation was undertaken to elucidate the following questions: 1) Can hydralazine in the conventional formulation be administered b i d or in a q i d regimen necessary to control blood pressure? 2) Is a slow release formu-

lation of hydralazine preferable to the conventional formulation? 3) Does the acetylator phenotype of the individual patient influence BP control?

METHODS AND PATIENTS

The trial

Using a double blind cross-over design hydralazine b.i.d. (A) hydralazine q.i.d. (B) and slow release hydralazine b.i.d. (C) were compared for efficacy and tolerability. The trial comprised three two-week periods and the patients were randomized to one of the six treatment sequences ABC ACB BAC BCA CAB and CBA.

Patients

Twenty out-patients (13 men, 7 women) attending our Hypertension Clinic were included in and completed the trial. Their median age was 59.5 years (range 41-75), median height 170.5 cm (range 154-187) and median weight 77 kg (range 48-110). The median duration of antihypertensive treatment was 36 months (range 11-141) and the median duration of the pre-trial treatment was 3.5 months (range 2-30).

The following criteria for inclusion were applied: hypertension according to WHO stage I or II of benign, stable diastolic type and adequately controlled by a constant dose of hydralazine q.i.d. in combination with a β -blocking agent and/or methyl dopa as well as diuretics for at least two months. Excluded were patients with congestive heart disease, valvular heart disease, bradycardia with a heart rate at rest of less than 40 beats/min, bronchospastic disorders, renal insufficiency, collagen disease, insulin dependent diabetes mellitus and hypertensione retinopathy of grades III and IV.

Medication

Double blind double-dummy technique was employed using tablets of identical appearance (Apresoline® 25 or 40 mg, Apresoline® Retard 40 mg and placebo) delivered in weekly dispensers so that the number of doses and the appearance of the medication were constant during the three treatment periods. Tablets were taken at 8 a.m., 16 and 10 p.m.

Ten patients received 200 mg and ten 100 mg of hydralazine per day. Seventeen patients received either metoprolol or propranolol. Ten patients received methyl dopa. The dose of concomitant medication was kept constant during the whole trial.

Parameters

The patients were evaluated during the last two days of each of the three two-week periods. All efficacy criteria were recorded once an hour from 8 a.m. until 6 p.m. They included systolic and diastolic BP, supine and standing as well as pulse rate, supine and standing. Korotkov sound phase V was used as the diastolic BP. The measurements were made by three observers (medical students) who were carefully instructed. A conventional sphygmomanometer was used with a cuff to suit the circumference of the arm.

Tolerability was recorded on the first of the two evaluations.

Routine laboratory tests (ESR, hemoglobin, creatinine, alanine aminotransferase, alkaline phosphatase, Na and K in serum) were performed by allocation and at the end of each treatment period.

Acetylator phenotype was determined in all before entering the trial using the sulfadimidine test (7). Ten patients were slow and nine fast acetylators, five were slow/fast acetylators.

Statistical methods

Parametric analysis of variance was used. The treatments were compared for variation during the trial as well as for overall mean values of BPs and pulse rate. 11 supine BP values during the day in each period based on means of 2x2 values i.e. two every hour on two days. Similarly the standing pulse rate were based on means of 1x2 values i.e. determination every hour on two days. The systolic variation between first and second supine BP measurement on the same day was tested. The appropriateness of using mean values was confirmed. The systematic differences between the first and the second day's BP and rate were also tested. Analyses based on the second data compared to the means did not reveal any differences.

The assumption of homogeneity of variance held data. Assumptions of normal distributions of data not always met but violations were moderate and ably unimportant.

Tests for residual effects as well as period effects made with the Koch (21) and Grizzle (13) models.

For 11 patients all measurements were made by the same observer, for seven patients by two and for two patients by three observers. The variation due to observers proved to be negligible (χ^2 test, analysis of variance and χ^2 goodness of fit on digit preferences).

The BPs and pulse rates were tested for relation to the acetylator phenotype (parametric analysis of variance, Bartlett's test). The statistical analysis was performed by B. Andersen.

RESULTS

In no case were even non significant tendencies towards either period effects or carry-over effects found.

Supine blood pressure

Diurnal variation is shown in Fig. 1. No significant differences were found between the three treatments, either for systolic or diastolic BPs ($p > 0.1$), though there was a non significant tendency for diastolic pressure to be lower late in the afternoon on hydralazine q.i.d. Significant variations during the day were present on all three treatments.

Table 1 Supine blood pressure and pulse rate (mean values of 20 patients day means)

hydralazine b i d B=hydralazine q i d C=slow release hydralazine b i d						
	A	B	C	Differences of means		
				A-B	A-C	C-B
Blood pressure (mmHg)						
Overall mean	175.2	169.5	171.9	5.7	3.2	2.5
S.D.	26.1	22.8	19.1			
Range	137.4-238.2	130.8-211.5	140.7-203.1	(-3.9-+15.3)	(-3.4-+9.9)	(-3.3-+8.2)
Pulse rate (beats/min)						
Overall mean	98.2	97.1	97.7	1.0	0.5	0.5
S.D.	9.6	13.4	12.3			
Range	79.5-116.0	71.5-122.8	79.4-124.4	(-3.6-+5.6)	(-3.6-+4.5)	(-3.5-+4.5)
Standing blood pressure						
Overall mean	64.9	65.1	64.7	-0.2	0.2	-0.4
S.D.				(-1.8-+1.3)	(-2.1-+2.5)	(-2.3-+1.4)
Range				-0.2	0.0	-0.2
				(-2.1-+1.6)	(-2.4-+2.4)	(-2.3-+1.9)

95% Confidence limits in parentheses

highest values in the morning and the evening ($p < 0.01$)

The day means for supine BP are given in Table 1. Although BPs were lower on hydralazine q.i.d. than on the other two treatments, the differences were not statistically significant ($p > 0.10$).

Standing blood pressure

No statistically significant differences were found between the three treatments, either for diurnal variation or for the day means ($p > 0.10$).

Postural blood pressure alteration

There were no differences between the treatments ($p > 0.10$) for systolic BP but not for diastolic BP. There was a significant postural BP reduction ($p < 0.01$).

Supine and standing pulse rate

Diurnal variation is shown in Fig. 2. No significant differences were found between the three treatments ($p > 0.10$). Significant variations during the day were found irrespective of treatment, with increased values after noon corresponding to the decrease in BP ($p < 0.01$).

The day means for pulse rate are given in Table 1. There were no significant differences between the treatments ($p > 0.10$).

Postural pulse rate alteration

There were no statistical differences between the treatments ($p > 0.10$) and each was associated with a significant increase in pulse rate from supine to standing position ($p < 0.01$).

BLOOD PRESSURE (mmHg)

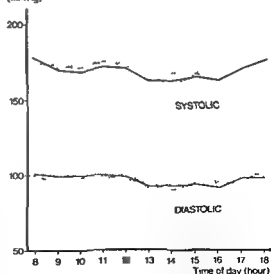


Fig. 1 Diurnal variation of supine BP ($p > 0.10$). — = hydralazine q.i.d. --- = slow release hydralazine b.i.d.

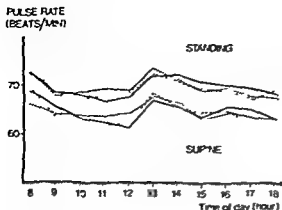


Fig. 2 Diurnal variation of pulse rate ($p > 0.10$). Symbols as in Fig. 1

Acetylator phenotype

There were no significant relationships in the treatments between acetylator phenotype and BPs or pulse rates in terms of diurnal variation or day means. There was however a tendency for the variability of systolic pressure to be greater and pulse rate lower in fast acetylators.

Tolerability

Three patients reported unwanted effects on conventional hydralazine. One had chronic headache, one had more sporadic headache and one had palpitations and tiredness. The laboratory investigations did not show any notable differences during the trial.

DISCUSSION

Hydralazine has a plasma half life of 2–3 hours (37). This has contributed to the current administration of four doses per day in the treatment of arterial hypertension. Studies in rodents however indicate that hydralazine is rapidly taken up and retained by the arterial blood vessels but slowly released (20). Furthermore, some metabolites of hydralazine are reported to be potent relaxants of vascular smooth muscle *in vitro* (28). There is consequently some basis for the experience that the antihypertensive effect lasts longer than one would expect from the plasma levels of the parent drug (28–38).

A few studies have compared two and four daily doses of hydralazine in conventional tablets. O'Malley et al (31) found identical BPs with b.i.d. and q.i.d. dosage regimens but the periods lasted only four days and only four patients were investigated.

In a multicenter study of 153 patients (23) which included patients from smaller series (1) no significant differences were found in BP or rate but side-effects were more frequent on than q.i.d. dosage. Some of the patients were drawn from the b.i.d. regimen due to inadequate control of hypertension or side-effects. All above studies were performed in an open fashion.

Other investigators (9, 12, 32) who conducted comparative studies have found quite antihypertensive effect of hydralazine b.i.d. dosage regimen.

Present experience with the slow release formulation of hydralazine is limited. The plasma concentration curve after single dose administration shows a lower peak compared with the conventional tablets (38, 39, 41). The maximum concentration is about 35% of that obtained with the conventional tablet and the peak appears after 1.5–3 hours against 1–1.5 hours. Conflicting results were obtained by Henningsen (16) who observed a peak with slow release tablets but this may be due to the fact that his patients had renal insufficiency or liver disease.

Talseth et al (39) performed an open study in which eight hypertensive patients previously treated with conventional hydralazine q.i.d. crossed over to slow release hydralazine b.i.d. identical daily doses. There was no significant difference in BP measured in the morning at six intervals although a small additional decrease in mean arterial BP was found. The authors suggest that this decrease might be due to improved compliance. Henningsen (17) compared the hydralazine preparations in an open study in 16 patients and found no significant differences in BP and pulse rate between conventional tablets 2–4 times daily and a slow release preparation in either one or two daily doses.

From this scant experience it is not possible to evaluate the role of slow release hydralazine in the treatment of arterial hypertension. The present investigation was performed with a randomized double blind technique and furthermore BP was measured hourly for 11 hours on two days in the treatment period. This yielded a total of 2640 observations for each criterion. Thus we obtained a reliable estimate of BP regulation with conventional hydralazine and slow release preparation, respectively and the significance of two- or four-dose administration. We found no statistically significant

differences in either BP regulation or pulse rate for the two preparations in terms of variations during the day as well as means for the day but there was a tendency for BPs to be lower on conventional hydralazine q.i.d. than on the other treatments. Confidence limits indicate that it is unlikely that a switch from conventional hydralazine q.i.d. to slow release or conventional formulation b.i.d. will increase day means of systolic/diastolic BP by more than 8/2/4.5 and 15/3/5 mmHg respectively. Similarly it is unlikely that BP will decrease by more than 3/3/3.5 and 3/9/3.6 mmHg respectively. Whether this has clinical significance is a matter of judgement.

Although the majority of patients show only insignificant differences in BP during the three treatments, some patients may respond unacceptably to change from a 4-dose to a 2-dose regimen. On the other hand, such a switch will probably improve BP control in some cases via better patient compliance. The current investigation could not elucidate compliance because with the double dummy all patients received four daily doses.

Side-effects are relevant to the value of hydralazine. With the introduction of β adrenergic blockers as a basic treatment in combination with hydralazine the side effects caused by hypertension are no longer a problem. Side effects reported by our patients were so few as to lack importance. The laboratory tests gave no indication of late toxicity but the study period is too short to elucidate this problem. It is well known that the acetylator phenotype of the patient has some influence on the occurrence of late toxicity of hydralazine. Thus hydralazine induced systemic lupus erythematosus occurs primarily in slow acetylators (33-35) and these patients have a higher concentration of hydralazine in plasma and a better BP control. We therefore examined the acetylator phenotype in our patients with special reference to BP control and side effects. No influence of the acetylator phenotype was found although there was a tendency to greater variability in systolic BP and a lower pulse rate in fast acetylators. Whether the frequency of late toxicity of hydralazine will increase with slow release formulation remains to be evaluated in long term studies.

From our short term study we conclude that conventional hydralazine used q.i.d. can in most patients be replaced by the same preparation or slow release preparation in b.i.d. dosage without impair-

ing BP control. In a minority of patients, however, BP may increase unacceptably on a b.i.d. regimen. For these patients we recommend a four-dose scheme.

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QT_c Intervals at Discharge after Acute Myocardial Infarction and Long-Term Prognosis

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ABSTRACT QT_c intervals were measured retrospectively in 463 survivors of AMI with a mean age of 59 years. The measurement was made once at discharge from hospital. Patients with anterior infarcts had significantly longer QT_c intervals than those with inferior or uncertain infarct localization. A weak but significant correlation was found between S-GOT maximum and QT_c interval. Patients with ventricular arrhythmias in the CCU had longer QT_c intervals. Patients with a poor long term prognosis had significantly shorter QT_c intervals. This finding was explained by digitalis therapy. Among patients with left bundle branch block, digitalis and quinidine, those below 66 years of age who died within the first 18 months tended to have longer QT_c intervals than the survivors. It is concluded that measurements of QT_c interval at discharge have no long term predictive value. This factor may, however, have some bearing on the short term prognosis in younger patients without therapy which affects the QT_c interval.

Key words: QT interval, acute myocardial infarction, prognosis.

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Prolongation of the corrected QT (QT_c) interval may be associated with an imbalance of the sympathetic nervous system (22-24, 29), cardiac disease (25), electrolyte disturbance (16, 27) and drug therapy (19). Ventricular fibrillation (VF) and sudden death have been described in two congenital syndromes with QT_c prolongation (15, 20, 23, 28). Recently studies have appeared on QT_c intervals in patients with ischemic heart disease (IHD). Patients with acute myocardial infarction (AMI) showed QT_c interval prolongation during the acute phase in two investigations (1, 8). A relationship between QT_c prolongation measured in the first ECG on admission to a coronary care unit (CCU) and malignant intraventricular arrhythmias during the CCU stay seems to exist in patients with AMI (2). Furthermore, sur-

vivors of out-of-hospital VF with coronary heart disease have been shown to have longer QT_c intervals than ambulatory post-MI patients without VF (10) and survivors of MI with a constant QT_c interval prolongation have been reported to be at high risk of sudden death (25).

The aim of this retrospective investigation was to study the QT_c interval at discharge after an AMI and to evaluate its clinical and long term prognostic implications.

PATIENTS AND METHODS

From 1968 to 1970 475 patients with AMI were treated in the CCU at Serafimerlasarettet and discharged alive. The routines of the unit, criteria for admission and diagnosis as well as a description of the patient group, have been published earlier (6, 12). Previous diseases, physical findings, ECG signs as well as complications in the CCU were registered on special charts and transferred in punch cards for computer analysis (9).

Three patients who had pacemakers and nine who could not be traced at the follow up in Dec. 1973 were excluded. The study thus included 463 patients: 304 men (66%) and 159 women (34%) with a mean age of 65 years (range 39-93).

Patients without bundle branch block (BBB), digitalis or quinidine therapy—factors known to influence the QT interval (16)—were studied separately. These 210 patients will be referred to below as the subgroup.

The mortality was evaluated on Dec. 31, 1973. 3-6 years after admission for the index infarction. Classification of modes and causes of death was based upon data from death certificates and hospital records. The routine police records of patients who had died outside hospital were checked for relevant information.

The QT interval was measured on the ECG recorded at

Abbreviations: QT = corrected QT interval; MI = myocardial infarction; AMI = acute MI; VF = ventricular fibrillation; IHD = ischemic heart disease; CCU = coronary care unit; BBB = bundle branch block; VEBs = ventricular ectopic beats; VT = ventricular tachycardia.

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Table I Mean QT_c (sec) at discharge in relation to previous diseases

Previous diseases	All patients (n=463)			Subgroup (n=210)		
	%	Mean QT _c	p	%	Mean QT _c	p
MI	24	0.426	NS	18	0.428	NS
No MI	76	0.426		82	0.431	
Angina pectoris	61	0.425	NS	57	0.429	NS
No angina pectoris	39	0.430		43	0.435	
Heart failure	29	0.414	<0.001	12	0.424	NS
No heart failure	71	0.432		88	0.432	
Hypertension	30	0.428	NS	20	0.435	NS
No hypertension	70	0.426		80	0.429	

discharge usually towards the end of the third week. From a 12 lead resting ECG at a speed of 50 mm/sec. the QT and RR intervals were measured in 3-5 consecutive beats. The values were averaged and QT was calculated from the mean value according to Bazett's formula (5, 16)

$$QT_c = \frac{QT}{\sqrt{RR}}$$

The measurement was made in the ECG lead with the longest QT interval. Care was taken not to measure QT intervals preceded by premature beats or to include U waves in the QT interval. The QT interval was measured in hundredths of a second from the beginning of the QRS complex to the end of the T wave where its hind limb joined the base line. Differentiation between T and U waves or some other potential was usually possible in at least one lead. If this was not possible the QT interval was measured from the beginning of the QRS complex to the notch between the T and U waves (16). All measurements were made by one of us (S.A.) without knowledge of the patients' clinical data.

Definitions

Previous myocardial infarction was accepted when reported by a patient and preferably verified by hospital records or ECG findings. *Previous heart failure* a history of digitalis therapy or diuretic therapy not given for hypertension. *Hypertension* a history of high blood pressure treated with antihypertensive agents. *Acute myocardial infarction and angina pectoris* were diagnosed according to conventional criteria (12, 21). *Left heart failure* was diagnosed by the presence of basal pulmonary rales or pulmonary vascular congestion on X-ray. *Site of infarct* anterior if the ECG criteria for infarction were present in two or more of leads CR₁, CR₂, and CR₃ and inferior if in two or more of leads II, III and aVF. Combination sites were determined according to the same criteria. In the following presentation ECGs with anterolateral sites were classified as anterior and inferolateral as inferior. Combined locations included antero (lateral) inferior infarcts. ECGs with signs of subendocardial infarcts or with BBB were classified as equivocal. *Sudden death* was defined as death within 2 hours after onset of the final symptoms.

Statistical methods

Conventional statistical methods were used. The influence of differences between the QT_c of each patient group was tested by Student's *t* test.

RESULTS

Clinical Features in Relation to QT_c at Discharge, Age, sex and previous diseases

QT_c intervals were not significantly related to age or sex either in the entire patient group or in the subgroup and were not longer in patients with previous MI, angina pectoris or hypertension. However, patients with a history of heart failure had significantly shorter QT_c intervals than the other. This difference was not noted in the subgroup (Table I).

Infarct site, size and complications

Patients with anterior infarcts had significantly longer QT_c intervals than those with inferior infarcts or infarcts of uncertain localization. This was true also in the subgroup (Table II).

Patients with high S-GOT maximum (normal value <40 U/l) had significantly longer QT_c intervals than those with lower values (Table III) and a similar non significant tendency was registered in the subgroup. This is also reflected by a weak significant correlation between S-GOT maximum and QT_c intervals ($r=0.13$, $p<0.01$) in the entire patient group. Patients with anterior infarcts had significantly higher S-GOT mean values than those with uncertain site of the infarct ($p<0.001$). No such difference existed between patients with anterior and inferior infarcts either in the total patient group or in the subgroup.

Table II Mean QT_c (sec) at discharge in relation to site of index infarct

% of infarct	All patients (n=463)		Subgroup (n=210)	
	%	Mean QT	%	Mean QT _c
anterior or anterolateral	32	0.437	34	0.445
anterior or inferolateral	25	0.424	36	0.419
lateral	4	0.438	6	0.433
combined	3	0.424	3	0.446
certain	35	0.417	21	0.426

$p < 0.05$ * $p < 0.01$ *** $p < 0.001$

QT_c intervals in patients with signs of heart failure in the CCU did not differ from those in patients without heart failure. There was no relationship between heart size measured by chest X-ray at discharge and QT_c interval.

Patients with ventricular ectopic beats (VEBs) in the CCU had significantly longer QT_c intervals than others (Table III). Patients with ventricular tachycardia (VT) (≥ 3 VEBs in succession) also had significantly longer QT_c intervals. Nine patients had developed VF in the CCU. Their QT_c was 439 ± 0.037 sec (mean \pm S.D.) and somewhat longer than the QT_c of patients with VT but it did not differ significantly from that of the remainder (426 ± 0.043 sec).

Indle branch block, digitalis and quinidine therapy

The mean QT_c of the 210 patients in the subgroup without BBB, digitalis or quinidine was 431 ± 0.034 sec. Nine patients had BBB but were not on digitalis or quinidine. Their QT_c was 472 ± 0.039 sec and differed significantly from that of the subgroup ($p < 0.001$). Fifty-three patients were treated with quinidine but had neither BBB nor digitalis therapy. Their mean QT was 0.454 ± 0.047

sec and significantly prolonged (5%) compared with the subgroup ($p < 0.001$). A large number of patients were on digitalis ($n = 152$) but they did not have BBB or quinidine therapy. Their mean QT_c was significantly shorter (6%) than that of the subgroup (0.403 ± 0.039 vs 0.431 ± 0.034 sec $p < 0.001$). No significant difference in any of the parameters presented in Tables I-IV was found among the digitalis-treated patients. Regarding all these parameters, the patients on digitalis had significantly shorter QT_c intervals than those in the subgroup.

Follow up

During the follow-up period 177 patients (38%) died. Their mean age at death was 69 years (range 45-93). The mean QT_c at discharge of the 286 survivors was 0.431 ± 0.043 sec, significantly longer than that of the non-survivors (0.419 ± 0.042 sec $p < 0.01$) (Table IV). Almost identical values were however noted between survivors and non-survivors in the subgroup (Table IV).

Of the 286 survivors 41 were on quinidine only (mean QT_c 0.454 ± 0.044 sec) and eight on both digitalis and quinidine (mean QT_c 0.426 ± 0.029 sec). Fifty-three patients (19%) were on digitalis

Table III Mean QT_c (sec) at discharge in relation to S-GOT_{max} and complications during the CCU period

	All patients (n=463)			Subgroup (n=210)		
	%	Mean QT	p	%	Mean QT	p
GOT _{max} < 200 μ l	77	0.423	< 0.01	80	0.429	NS
GOT _{max} > 200 μ l	23	0.436		20	0.439	
right heart failure	67	0.427	NS	54	0.432	NS
left heart failure	33	0.424		46	0.429	
EBs	87	0.429	< 0.01	86	0.433	< 0.01
> 3 VEBs	13	0.410		11	0.416	
VT	40	0.434	< 0.01	36	0.435	NS
> 3 VT	60	0.421		64	0.428	

Table IV Mean QT_c (sec) at discharge in relation to survival and different modes of death during the follow up

	All patients (<i>n</i> =463)		Subgroup (<i>n</i> =210)	
	%	Mean QT_c	%	Mean QT_c
Survivors	62	0.431	78	0.431
Deceased	38	0.419**	22	0.430
Mode of death				
Sudden <2 h	7	0.424	5	0.420
IHD 2-24 h	8	0.424	5	0.444
IHD >24 h	13	0.413	8	0.428
Not IHD	9	0.414	5	0.427

* $p < 0.05$ ** $p < 0.01$

only and had significantly shorter intervals than the other survivors (mean QT_c 0.403 ± 0.043 vs 0.437 ± 0.041 sec $p < 0.001$)

Twelve of the 177 patients who died were on quinidine only (mean QT_c 0.452 ± 0.045 sec) and ten on both digitalis and quinidine (mean QT_c 0.421 ± 0.043 sec). Ninety three (53%) of those who died were on digitalis only. Their mean QT_c was significantly shorter than that of the other non survivors (0.403 ± 0.039 vs 0.437 ± 0.041 sec $p < 0.001$)

The relation between QT_c interval and mode of death is shown in Table IV. Sixty nine patients (15%) died of IHD within 24 hours after onset of the final symptoms. Their mean QT_c value did not differ significantly from those who died of IHD 24 hours or more after onset of symptoms or from those who died from other causes than IHD. Forty nine patients (11%) died within six months, 28 (6%) 7-12 months and 100 (22%) more than 12 months after discharge. These three groups of deaths did not differ significantly as to their mean QT_c intervals nor were any differences found within these groups when QT_c intervals were related to the mode of death. Separate analysis of the subgroup also failed to show any relationship between QT_c interval and time and mode of death.

However, among patients below 66 years of age ($n=237$) those who suffered a cardiac death within the first follow up year ($n=21$) tended to have longer QT_c intervals than the survivors (0.432 ± 0.044 vs 0.427 ± 0.040 sec N.S.). This tendency was also found when only cardiac deaths ($n=14$) during the first six months were considered (0.434 ± 0.041 vs 0.426 ± 0.040 sec N.S.). Among subgroup patients below 66 years of age ($n=139$)

the difference in QT_c interval between those died within six months after discharge ($n=7$); the survivors reached an almost significant level (0.440 ± 0.042 vs 0.427 ± 0.032 sec). Analysis of these two groups below 66 years regarding parameters presented in Tables I, II and III show the same tendencies as in the corresponding group without age limit.

VT had been registered in 185 patients in CCU, 123 (66%) of them survived the follow up period and their QT_c value was 0.436 ± 0.040 sec compared to 0.431 ± 0.046 sec in 62 patients VT who died. There was no association between QT_c interval and time and mode of death in this group. However, three of the subgroup patients with VT who died within the first six months had prolonged QT_c intervals (0.483 ± 0.040 vs 0.433 ± 0.037 sec $p < 0.05$).

A QT_c interval of 0.440 sec is often used as a normal upper limit. 143 (31%) of our patients had QT_c intervals exceeding this value. A QT_c interval above 0.440 sec was not associated with increased mortality.

DISCUSSION

Simple methods to identify high risk patients after an AMI are needed for intervention studies. This report presents the long term prognostic importance of QT_c at discharge from hospital. So far, the study of Schwartz and Wolf (25) in which repeated QT_c measurements were performed during a one year follow up in 55 post MI patients is only one reporting a prognostic significance of prolonged QT_c intervals in survivors of AMI. The authors who did not include patients with BBE, digitalis induced changes that obscured the ECG analysis in their study population found that 28 patients who died, all of them suddenly, had significantly longer QT_c intervals than the survivors and the matched controls. Contrary to their results, we found that patients who died had significantly shorter QT_c intervals than the survivors. This is explained by the digitalis therapy in this group of patients with congestive heart failure and accordingly poor prognosis. This finding of drug influence on QT_c points to the difficulty in using this interval in prognostication. Schwartz and Wolf did not discuss in detail possible drug influence on QT_c . On the other hand, the influence of drugs may be

are less important in their fairly young patient group (mean age 52 years)

More in agreement with the findings of Schwartz and Wolf, our patients below 66 years of age who died within the first follow up year and especially those who died in the first six months had a tendency to longer QT_c intervals at discharge than the corresponding survivors. This was even more marked in patients without BBB or digitalis and amiodarone treatment. In another study in which the same age selection was used (1) we found an association between prolonged QT_c intervals at discharge and reinfarction and sudden death in survivors of AMI. In that study we measured QT_c intervals on several occasions during the follow up period. It is possible that a constant QT_c prolongation increases the prognostic weight of this parameter since patients with normal mean QT_c (below 0.44 sec) occasionally had a prolonged QT_c interval which was found also by Schwartz and Wolf.

QT_c prolongation during the acute phase of AMI has recently been reported (1, 8). On the contrary our report deals with the measurement of QT_c interval during the subacute phase of AMI. Our findings indicate that a history of previous MI does not influence the QT_c interval after AMI in contrast to the extent of myocardial damage during the recent AMI. Thus we found a weak correlation between

GOT maximum and QT_c at discharge. This does not agree with the results of Doroghazi and Childers (1) who found no association between peak creatine kinase values and the presence of transient QT_c lengthening beyond the normal limit during the hospital stay in patients with AMI. From our results it is obvious that there are other factors which influence the QT_c interval as our patients with anterior infarcts had longer QT_c intervals than those with inferior infarcts though there was no significant difference between them in S-GOT maximum. The causes and implications of this finding are uncertain. The difference might to some extent be explained by the method of ECG registration with more extensive changes in the precordial leads facing the anterior wall of the left ventricle than in the standard leads reflecting the inferior one.

Prolongation of the QT_c interval is sometimes seen in patients with the billowing mitral valve syndrome (3, 4, 18) and may be of relevance to its association with malignant ventricular arrhythmias and sudden death (26). This relationship between QT_c prolongation and malignant ventricular ar-

rhythmias also exists in certain hereditary syndromes (15, 20, 28). Our patients with ventricular arrhythmias during the CCU stay had longer QT_c intervals at discharge from hospital than those without these arrhythmias. In patients with all forms of VEBs including VT and VF this difference was not explained by the effect of therapy. This association between QT_c prolongation and ventricular arrhythmias in patients with IHD is also reflected by the report of Haynes et al. (10) who found repolarization abnormalities including QT_c prolongation in survivors of out-of-hospital VF. In a previous study we found an association between QT_c prolongation on admission in patients with AMI and malignant ventricular arrhythmias during the acute phase (2).

Other factors previously found to be related to changes in QT_c include hypertension (11, 16) but no such relationship was evident in our patients. It may be that the recent infarction had a dominant influence on the QT_c interval (1, 8). In contrast to the findings of Hegglin and Holzmänn (11) prior heart failure was related to a short QT_c interval in our patients. This may be explained by the shortening effect of digitalis on QT_c. However at discharge most patients who had shown signs of heart failure in the CCU were not on digitalis but on diuretics alone. These drugs will not influence the QT_c interval unless electrolyte disturbances are induced (27) and no patient showed such imbalance at the time of discharge.

The present findings indicate that the QT_c interval measured at discharge from hospital is associated with the extent of the recent myocardial damage, the site of the infarct and ventricular ectopic activity during the CCU period. Our data also indicate that the QT_c interval as such measured once in the subacute phase of the illness hardly has any bearing on the long term prognosis. We found that drug therapy—in this study digitalis—interferes to a considerable extent with the evaluation of QT_c as a prognostic indicator. There was however a tendency to QT_c interval prolongation in younger patients not on drugs affecting QT_c and without BBB dying within the first half year after their AMI. Several studies have pointed to the feasibility of estimating prognosis after an AMI (7, 12, 17). However a number of patients dying early after discharge carry none of the ordinary unfavourable prognostic signs (14). This warrants a search for new prognostic factors that may increase our pre-

dictive ability. It has been suggested that repeated assessments of a prognostic factor may improve their predictive value (13). This has been done with QT_c interval by Schwartz and Wolf (25) with promising results. The prognostic implications of the QT_c time in combination with other prognostic factors remain to be evaluated and will be discussed in a further report.

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Antiarrhythmic Properties of a Neuroleptic Butyrophenone, Melperone, in Acute Myocardial Infarction

A Double Blind Trial

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ABSTRACT In vitro animal studies suggest melperone, a neuroleptic butyrophenone, to be a type III antiarrhythmic drug according to the classification of Vaughan Williams. It has no negative inotropic effect on cardiac muscle. A double-blind trial of 3 hours duration was carried out with melperone and placebo in 26 patients admitted to the CCU with suspected acute myocardial infarction (AMI) and ventricular rhythms. Melperone, 50 mg i.v. was superior to placebo in reducing the total number of ventricular ectopic beats (VEB) as well as the number of minutes with either frequent, multifocal, R on T type or runs of VEB. The reduction became statistically significant in the second treatment hour in patients with definite AMI. Melperone induced sedation and reduction of systemic BP in most of the patients. Two patients with low initial systolic BP achieved a further reduction and had BP levels below 90 mmHg. Two experienced minor side-effects. In conclusion, melperone administered in large i.v. doses to patients with AMI induced sedation, acute BP reduction and some reduction of ventricular arrhythmia.

Key words: myocardial infarction, arrhythmia, neuroleptic, continuous ECG.

Acta Med Scand 208 61 1980

The high incidence of ischemic heart disease makes it necessary to search for neuroleptics (major tranquilizers) without harmful side-effects on the heart circulation. Melperone is a neuroleptic butyrophenone which differs from high-dose neuroleptics of the phenothiazine type in having no anticholinergic effect (2). Studies on isolated guinea pig papillary muscles have shown differences among neuroleptics concerning their influence on the action potential. Only melperone prolongs the

action potential without affecting the contractile force (1). This indicates antiarrhythmic properties of type III according to the classification of Vaughan Williams (11).

In a preliminary unpublished study, melperone given i.v. to patients suffering from acute myocardial infarction (AMI) was well tolerated and seemed to reduce the number of ventricular ectopic beats (VEB) when doses exceeded 20 mg. In the present study the VEB-reducing effect of melperone is evaluated against that of placebo in patients with suspected AMI and ventricular arrhythmias.

PATIENTS AND METHODS

Twenty-three males and three females admitted to the Coronary Care Unit (CCU) with suspected AMI entered this study. Table I gives relevant data for the entire series. Myocardial infarction was diagnosed according to the WHO criteria (12) in 16 patients. 10 were assigned to the non-infarction group.

Ventricular arrhythmia was defined according to the following criteria: **VEB** Premature QRS complex with a duration of more than 0.10 sec and a configuration different from the regular QRS complex. **Monofocal VEB** VEB of similar configuration and constant coupling time to the normal complex. **Multifocal VEB** VEB of different configurations. **R-on-T type VEB** VEB starting in a T wave. **Runs of VEB** 2-5 VEB in succession with a frequency of more than 100 beats/min. **Ventricular tachycardia (VT)** More than 5 VEB in succession with a frequency of more than 100 beats/min. The criteria for entering the trial were frequent VEB (more than 5 VEB/min) and/or multifocal VEB and/or runs of VEB and/or R-on-T type VEB.

Abbreviations: AMI = acute myocardial infarction, BP = blood pressure, VEB = ventricular ectopic beats, VT = ventricular tachycardia, VF = ventricular fibrillation, CCU = coronary care unit.

Table I Distribution of patients completing the trial

	Proven AMI		Non proven AMI	
	Melperone	Placebo	Melperone	Placebo
No. of pats	9	5	5	5
Sex				
♂	8	3	5	5
♀	1	2	0	0
Age (y)				
Mean	66	67	68	66
Range	56-73	49-84	54-85	56-78
Infarction site				
Anterior	5	4		
Posterior	3	1		
Uncertain or combined	1	0		
Duration of symptoms before study				
<4 h	0	1		
4-24 h	4	3		
>24 h	5	1		
Cardiac enlargement (chest X-ray)	7	3	3	3
Pulmonary congestion (stethoscopic)	5	2	2	1
Previous myocardial infarction	1	1	4	3
Digoxin treated	1	3	1	3
Atrial fibrillation	1	1	0	0

Patients with VT, systolic blood pressure (BP) below 90 mmHg, frank pulmonary edema or bradycardia (heart rate below 50 beats/min) or any degree of sinoatrial or atrioventricular block were excluded.

All patients were ECG monitored continuously for 3 hours during their stay in the CCU (Fig. 1). The ECG recordings were performed with a Mingograph 61 (paper speed 10 mm/sec) connected to the central monitoring equipment using the same precordial lead and out of sight of the patient. The first hour served as a reference and no treatment was given. Heart rate, systemic BP by the cuff method, respiratory rate and general clinical condition were recorded. One hour after the start of the ECG monitoring, 50 mg of either melperone (Buronil®) or placebo (solvent alone) in a 10-ml injection in five divided doses were given every other min during a 10-min period. After each injection, heart rate, BP, respiratory rate and general condition were re-recorded. During the next two hours the patients were re-examined and interviewed according to a questionnaire regarding side-effects. The 180-min ECG strips were analysed blindly by manual counting of the ventricular arrhythmias according to the definitions.

Statistical analyses were made by Student's *t* test with the significance level chosen as $p < 0.05$.

RESULTS

The results are presented in Tables II and III.

In patients with AMI receiving melperone, number of minutes with ventricular arrhythmia (either frequent multifocal R on T type or run VEB) was significantly reduced in the second treatment hour. Furthermore, melperone produced a significant reduction of the most common type of arrhythmia (i.e. frequent VEB). Placebo did not alter significantly the frequency of arrhythmia in the AMI group. The frequency of arrhythmia in the non-infarction group seemed rather constant, being equally influenced by melperone and placebo.

Two patients with AMI, one receiving melperone and the other placebo, were excluded due to short lasting VT. None of the patients developed ventricular fibrillation (VF). No sort of block (sinoatrial, atrioventricular or bundle branch block) developed during melperone treatment. Two patients received submaximal doses of melperone.

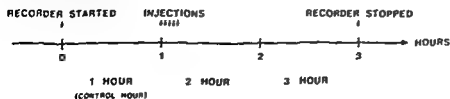


Fig. 1 Schedule of the trial

Table II Overall arrhythmia frequency (mean \pm S.E.M.)

The first hour served as a control hour after which the injections were given. The arrhythmias were analysed manually due to the different mean control values for melperone and placebo for patients with AMI; the statistical evaluation (Student's *t* test) was performed as an intra-group and not a between-group comparison.

	Proven AMI		Non proven AMI	
	Melperone (N=9)	Placebo (N=5)	Melperone (N=5)	Placebo (N=5)
Minutes with >5 VEB				
1st h	37.3 \pm 4.8	26.8 \pm 6.6	23.8 \pm 11.1	30.0 \pm 10.6
2nd h	22.7 \pm 6.8	18.4 \pm 8.8	15.5 \pm 8.9	27.6 \pm 10.9
3rd h	15.4 \pm 6.4*	14.4 \pm 10.1	21.6 \pm 11.0	25.0 \pm 11.9
Minutes with either >5 VEB multifocal VEB, run of VEB or R/T				
1st h	39.0 \pm 4.8	30.8 \pm 6.3	30.0 \pm 11.0	38.0 \pm 9.8
2nd h	24.7 \pm 6.4	22.2 \pm 8.0	26.8 \pm 9.9	35.6 \pm 11.8
3rd h	16.6 \pm 6.4*	16.4 \pm 9.3	25.8 \pm 11.4	31.4 \pm 12.7
EB (%)				
1st h	11.4 \pm 2.1	7.6 \pm 2.9	7.9 \pm 3.9	7.4 \pm 3.0
2nd h	6.0 \pm 1.5	5.1 \pm 2.3	7.5 \pm 4.4	7.0 \pm 3.1
3rd h	5.5 \pm 2.5	5.8 \pm 4.3	7.8 \pm 4.4	7.3 \pm 3.4

**p* < 0.01

0 and 40 mg) due to decreasing systolic BP from 0 to values below 90 mmHg. Melperone reduced significantly the systolic and diastolic BP. No significant change was found in the heart rate (Table I).

Of the melperone treated patients 11 were treated with maximum effect in the second post-injection hour and only 4 felt unchanged. Of the placebo-treated patients 9 felt unchanged and 2 experienced some drowsiness. Two melperone-treated patients complained of dizziness, one experienced dryness of the mouth and one complained about a general feeling of warmth. Otherwise no side-effects were recorded in either group.

Table III Changes in heart rate and BP from before injections to after the last injection 10 min later (mean \pm S.D.)

	Changes in heart rate (beats/min)	Changes in BP (mmHg)	
		Systolic	Diastolic
Melperone (N=15)	1.7 \pm 1.0	-15.3 \pm 13.1	-6.7 \pm 12.1
Placebo (N=11)	0.5 \pm 1.0	1.8 \pm 4.1	4.1 \pm 7.4
Value	0.77 (NS)	<0.001	0.02

DISCUSSION

Since the late 60s certain ventricular arrhythmias have been considered as warning signals of primary VF in myocardial infarction (7). It has been generally accepted that these arrhythmias should be treated in order to prevent VT and VF. However some investigators found that 50% of all primary VFs occur without warning arrhythmias (4, 5, 6). Therefore studies on antiarrhythmic drugs should concentrate on the prophylaxis of VF in all patients suspected of AMI.

We tried to evaluate the effect of melperone on warning arrhythmias. Even well trained nurses detect only a small fraction of ventricular arrhythmias on a monitor scope (8). Computer based arrhythmia detection is also associated with serious drawbacks (3). Therefore we used manual evaluation of continuously recorded paper ECG during a 3 hour period.

Due to the variation in occurrence of arrhythmia in intermittent ECG sampling (Fig. 2 and ref. 10) we decided to use a 60-min period as control before treatment. Since VT must be considered a real threat of VF, patients with such episodes were excluded.

Melperone had some antiarrhythmic effect on our AMI patients. This effect appeared however late after the injection, reaching statistical significance

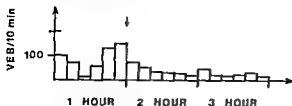


Fig 2 Spontaneous changes in ectopic activity in a patient during the observation hour. Arrow indicates melperone injection.

only in the second postinjection hour. Only one of ten patients became completely free from warning arrhythmias. The results may not be applicable to the very first hours of myocardial infarction because most of our patients had infarcts of 4–24 hours duration.

The assumption that melperone should act as a type III antiarrhythmic drug is not supported by the present findings. It is likely that the antiarrhythmic effect is mediated via the central nervous system and is also separated from the peripheral α blocking effect. The injection was immediately followed by a decrease in BP; the antiarrhythmic action was coincident with the peak of the sedative effect and the antiarrhythmic effect was very weak when infarction was absent. The absence of antiarrhythmic effect of melperone in the non-infarction group might reflect a difference in arrhythmia triggering mechanisms in the infarction and non-infarction groups (9). The stable frequency of arrhythmia in the non-infarction groups might be due to a lesser susceptibility to a varying degree of stimulation of the autonomic nervous system. Melperone produced a decrease in BP within 10 min after injection. This effect may be beneficial when hypertensive patients are treated but deleterious in infarct patients who already have a low BP.

In conclusion, large i.v. doses of melperone given to a small number of patients with AMI reduced warning arrhythmias. The acute BP reduction com-

bined with the slow onset of antiarrhythmic effects limits the use of melperone as an antiarrhythmic drug. Further studies are justified to see if intramuscular melperone in doses well known from psychiatric practice will influence arrhythmia and BAMI patients.

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Ventricular Arrhythmias During Exercise Testing and 24-Hour ECG Tape Recording in Patients with Ischaemic Heart Disease and in Normal Individuals

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ABSTRACT The occurrence of ventricular arrhythmias (VA) during resting ECG, maximal bicycle exercise testing and 24 hour ECG tape recording (24 ETR) was studied in 24 patients and 24 matched normal persons. The patients were on treatment with β -receptor blocking agents or verapamil for ischaemic heart disease (IHD) verified by coronary angiography. The matched persons showed no signs of heart disease. VA did not occur in any of those studied on a 60 second resting ECG. During exercise testing one patient and six normal subjects had VA. During 24 ETR, VA were found in 16 patients and 10 normal persons. The maximum heart rate during exercise and the average heart rate during ETR did not differ significantly between individuals with and without VA in the groups of patients and normal subjects, respectively. It is concluded that in IHD patients receiving β -blocking agents or verapamil, 24 ETR is a more reliable indicator of VA than exercise ECG testing. In normal individuals the two methods are of similar value.

Key words: ventricular arrhythmias, exercise testing, 24 hour ECG tape recording, ischaemic heart disease, normal individuals.

Acta Med Scand 208 65 1980

Ventricular arrhythmias (VA) increase the risk of sudden cardiac death both in persons without symptoms of heart disease (4-7) and patients with known ischaemic heart disease (IHD) (11-13, 14). A resting ECG rarely provides sufficient information about the occurrence of VA in the individual patient (9, 16). On the other hand, it is difficult at present to determine whether exercise testing or ECG tape recording (ETR) is the most suitable method for the demonstration of VA. Some investigators emphasize the value of exercise ECG in

patients with IHD (1, 5, 8) while others consider ETR to be the more suitable of the two methods (3, 12, 15, 17). However, several of the authors quoted consider the methods complementary.

In order to compare the usefulness of the two methods we studied—by means of resting ECG, maximal bicycle ergometer testing and 24-hour ETR (24 ETR)—the pattern of VA in normal subjects and patients with IHD verified by coronary arteriography.

STUDY POPULATION AND METHODS

The investigation comprises two age matched groups of men who were studied according to a predetermined plan. Their main characteristics are given in Table I.

The 24 normal subjects were outpatients and fulfilled the following criteria: No symptoms of cardiac disease, normal heart stethoscopy, normal resting 12 lead ECG, normal heart size (X-ray of the chest), normal blood pressure, no signs of ischaemia on the ECG during maximum exercise testing, no drug intake.

The 24 IHD patients were fully mobilized during hospitalization. All had severe angina pectoris which could not be satisfactorily treated with propranolol 320 mg/day or equipotent doses of other β -blocking agents or verapamil. A stenosis of 50% or more of at least one of the large coronary arteries had been demonstrated in the preceding 3 months by coronary arteriography in 11 of the patients. Fourteen patients had had acute myocardial infarction (AMI) at least 3 months before the present investigation. The antianaginous treatment had been gradually discontinued over a period of 3 days prior to the arrhythmia investigation.

A resting 12 lead ECG (I-II, aVL, aVR, aVF and V₁₋₆) was recorded for 60 sec with the patient in the supine

Abbreviations: VA = ventricular arrhythmias, IHD = ischaemic heart disease, ETR = ECG tape recording, 24 ETR = 24-hour ETR, AMI = acute myocardial infarction, VE = ventricular extrasystoles.

Table I Clinical characteristics of the two groups

	Normal group	IHD group
N	24	24
Age (y)		
Mean	53	54
S.D.	10	7
Previous AMI		14
Therapy prior to study		
Digoxin		3
β blocking agent		11
Verapamil		12
None	24	2

position immediately before start of the exercise testing. This was a symptom limiting bicyclic ergometer test carried out at a stepwise increasing work load. A 6-lead ECG (I-II, V_1 , and V_6) was continuously registered on a Mingograph III (Siemens Elema) from the start until 5 min after discontinuation of the test.

After 30 min rest the 24-ETR was commenced using 2 bipolar leads employing a Medilog tape recorder. The tape was analysed at 60 times the real speed on a Pathfinder high speed ECG analyser. All complicated arrhythmias were printed on paper using the real time. The number of QRS complexes and the number of ventricular extrasystoles (VE) were registered during the analysis. VA were classified in respect of all three methods of investigation according to Lown and Wolf (10): grade 0 no VE, grade 1 isolated unifocal VE, grade 2 unifocal VE numbering at least 1 VE/min or 30 VE/hour, grade 3 multifocal VE, grade 4a paired VE, grade 4b three or more consecutive VE, grade 5 R-on T phenomenon ($RR/QT < 1$).

Statistical methods

Student's *t* test was employed in the statistical analysis of the results for the comparison of the mean values and the χ^2 test for comparison of the frequencies. A significance level of 5% was chosen.

RESULTS

Resting ECG

VA did not occur in any of the subjects examined. There was no significant difference in the heart rate between the normal and the IHD group (Table II). Neither could any difference in the heart rate be demonstrated between IHD patients with and without previous myocardial infarction or between patients with β blocking or verapamil treatment.

Exercise testing

The normal subjects studied had a considerably better working capacity than the patients with IHD

(Table II). No difference in the maximum heart rate was found between IHD patients with and without previous myocardial infarction or patients who had received β blocking agents or verapamil. The only IHD patient with VA during the exercise testing had suffered an AMI three years before and was receiving no drugs. In the normal group 11 persons (25%) had VA in connection with the exercise testing. In all instances a few unifocal VE (Table III). The maximum heart rate was not significantly different in the normal subjects with and without VA. Ventricular arrhythmias mainly occurred just after the exercise testing. The exercise testing was usually performed in the morning.

24 hour ECG tape recording

The average duration of monitoring was equal in the two groups (Table II) and was carried out in cases between 0 and 6 a.m. In some cases monitoring was terminated before 24 hours due to planned coronary arteriography or because the patient was unable to return to the hospital at required time. The average heart rate did not differ significantly between the normal and the IHD group neither during the monitoring nor as a whole during the period 0-6 a.m. There was no difference in the heart rate between IHD patients with and without AMI neither in respect of the whole monitoring period nor the period 0-6 a.m. Similarly no difference in the heart rate could be observed between patients who had been on treatment with β blocking drugs or verapamil. VA occurred in 46% of the normal subjects and in 16 (67%) of the patients with IHD. This difference is not statistically significant. During the whole period of monitoring two persons in the normal group had multifocal VE while three patients in the IHD group had multifocal VE, paired VE or R on T phenomenon (Table III). During the period 0-6 a.m. VA grade 3-5 were seen in the IHD group only and in general the severity of arrhythmias was reduced during sleep in both groups (Table III). In the IHD group 10 of 14 patients with previous AMI and 10 without previous AMI had VA. The difference was not significant. No significant difference in the heart rate was observed in either group when comparing patients with and without VA. Similarly the heart rate of patients with VA during sleep did not differ significantly during this period from that of other patients.

II Resting ECG, exercise ECG and 24 ETR in the two groups studied (mean \pm S.D.)

	Normal group	IHD group	p value
Resting ECG			
Heart rate (min ⁻¹)	66 \pm 10	66 \pm 10	n.s.
Exercise ECG			
Max. heart rate (min ⁻¹)	166 \pm 22	118 \pm 24	<0.001
Total load (kpm)	9 172 \pm 5 300	3 330 \pm 2 400	<0.001
TR			
Duration (min)	1 279 \pm 202	1 190 \pm 146	n.s.
Heart rate (min ⁻¹)	78 \pm 9	73 \pm 11	n.s.
Heart rate at 0-6 a.m. (min ⁻¹)	63 \pm 8	66 \pm 10	n.s.
Mean VE range (hour ⁻¹)	0-33	0-40	

Exercise testing versus 24 hour ECG tape recording

VA occurred more frequently both in the normal subjects and in the IHD patients during ECG tape recording (Table III) but the difference is significant in the IHD group only. All patients with VA during exercise testing also had VA during tape recording. The five normal subjects with VA during tape were also among those six who had VA during exercise testing.

DISCUSSION

The incidence of VA in a person depends on the registration technique employed and the population studied. This investigation demonstrates, like others (9, 16) that resting ECG is insensitive in the detection of VA. It also shows that exercise testing and 24-ETR do not differ significantly in sensitivity regarding detection of VA in normal subjects. Multiple VA, however, were found only during tape recording. The incidences of VA of 25 and 46% re-

spectively are higher than in the two investigations employing the same methods on normal subjects (2, 12). Of 30 normal subjects of roughly the same age as those studied by us, 7% had VA during exercise testing against 33% during 24-ETR (12). Only unifocal VE were observed during exercise testing whereas more severe forms of VE were detected in four patients during tape recording. In a report comprising 12 patients VA occurred in two persons during exercise testing and in three during tape recording (2). The methods supplemented each other to a certain extent in another study (12) while the exercise testing in our study did not enhance the information obtained from the 24-ETR alone.

We found VA in our IHD patients significantly more frequently during 24-ETR than during exercise testing. The registered incidence of VA of 4% is considerably lower than that of 39-91% detected by exercise testing in other investigations comparing the two methods (2, 6, 8, 12, 15). The low incidence found in our study can be ascribed to the

Table III Incidence of VA during maximal bicycle ergometer test and 24 ETR in the two study groups

Maximal work load	Normal group			IHD group		
	24-ETR			24-ETR		
	Exercise ECG	Total period	At 0-6 a.m.	Exercise ECG	Total period	At 0-6 a.m.
18	13	19	23	8	14	
2	9	5	1	11	8	
4	0	0	0	2	0	
0	0	0	0	1	1	
0	0	0	0	1	0	
0	0	0	0	0	1	
0	0	0	0	1	0	

massive drug therapy continuing up to the period of monitoring. However, it is remarkable that such a pronounced antiarrhythmic effect on VA should be observed in patients under treatment both with β blocking agents and verapamil. The low incidence of VA might also be accounted for by a low maximum pulse rate during exercise.

The incidence of VA of 67% detected by 24 ETR corresponds roughly to that observed in other investigations by 24 hour monitoring, as the incidence of arrhythmias is stated to be 78–97% (2, 6, 12, 15), while complicated VA have been found by others in 37–49% (5, 12, 15) against 8% in the present investigation.

The current study shows that VA are detected in normal subjects more frequently by 24 hours of ambulatory ETR than by maximum bicycle ergometer testing, and thus that all persons with VA during maximum exercise testing also had VA during tape recording.

VE were registered significantly more frequently during 24 ETR than during exercise testing in patients with medicamentally intractable angina pectoris under treatment with either a β blocking drug or verapamil. Thus, it does not appear necessary to supplement 24-ETR with maximum ergometer bicycle testing for the evaluation of VA in patients with this disease.

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Episodic Cardiac Arrhythmia and Accident Rate

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ABSTRACT The rate of accidents severe enough to require a roentgen examination was investigated in a series of patients with episodic cardiac arrhythmia of types causing Adams Stokes syndrome. The accident rate was doubled compared with controls without own Adams Stokes syndrome but the associated increase in fracture rate was not significant.

Keywords: accident, Adams Stokes arrhythmia, fracture, trauma.

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In the past Adams Stokes syndrome was usually considered identical with third degree atrioventricular block. Today any arrhythmias—a bradycardia or a tachycardia—capable of causing cerebral symptoms ranging from mild dizziness to full blown syncope are being included (YHA Criteria Commission 1973). Recent studies have revealed that significant episodic cardiac arrhythmias are common among patients with dizziness and syncope (2, 5, 6, 7, 8, 9, 11). Unpredictable falling is a part of Adams Stokes syndrome. It has been shown that 13% of patients treated with pacemakers had been hospitalized because of trauma prior to recognition of the bradycardiac episodes (3). A group of patients treated for postapoplectic dementia who were proven to have occult episodes of severe arrhythmia also in most instances had sustained femoral neck fracture (1). The aim of this investigation was to estimate the risk of trauma and fracture in a series of patients with occult cardiac arrhythmias with cerebral symptoms in comparison with a control group selected at random.

STUDY POPULATION AND METHODS

A consecutive series of 330 patients referred to the Heart Clinic of the Medical Department due to suspected or likely episodic arrhythmia with Adams Stokes syndrome is examined with 24 hour ECG monitoring by means of

portable ECG recorders type SRA/HRB 2 (Helcomed Norden/Hellige). The period was extended to more than one day in borderline cases.

The tapes were processed by an automatic unit presenting all rhythms faster than 120 or slower than 40 beats/minute by single R-R interval detection as well as every 10th ventricular ectopic beat by configuration criteria as a one lead ECG on a paper strip. The ECGs were examined initially by a specialized laboratory technician and finally by one of us (N. J. A.).

Patients with minor arrhythmias such as isolated ectopic beats or sinus arrhythmia with normal rate were omitted from the group. The details of the heart examination have been presented elsewhere (1). Significant episodic arrhythmias—i.e. arrhythmias considered severe enough to cause cerebral symptoms—were present in 173 patients: 78 men (mean age 70 ± 14) and 95 women (mean age 72 ± 13). The types and distribution of arrhythmias are listed in Table 1.

The files of the radiology record room were searched for information about accidents leading to roentgen examinations during the 5 years preceding the diagnosis of arrhythmia. Since virtually all emergency roentgen examinations in the city are carried out in the Radiology Department of our hospital an almost complete coverage was attained of trauma episodes sufficiently serious to require a roentgen examination.

The following variables were recorded: 1) Number of patients who had had one or more roentgen examinations because of trauma. 2) Number of accidents which may be suspected of being caused by an episode of dizziness or fainting including falling from standing or sitting position, falling downstairs, falling off bicycles and similar incidents but excluding injuries obtained as passenger in a

Table 1 Diagnoses in 173 cases of significant episodic cardiac arrhythmia

	N
Supraventricular tachycardia including atrial fibrillation and flutter	29
Sick sinus syndrome—pure bradycardia	32
Sick sinus syndrome—brady tachy	36
Atrioventricular block 2nd degree	5
Atrioventricular block 3rd degree	24
Abundant ventricular ectopics or bigeminy	15
Ventricular tachycardia or coupled ventricular ectopics	32

Table II Accidents during five years in patients with arrhythmia and in age matched controls

	Patients			Controls		
	Men	Women	Total	Men	Women	Total
Total no. of persons studied	78	95	173	78	95	173
Patients with trauma roentgen	18	22	40	7	13	20
Fall accidents (total no.)	31	34	65	9	17	26
Fractures (total no.)	8	14	22	4	13	17
Femoral neck fracture	0	3	3	0	1	1

motor-car from beatings and similar accidents in which the patient was an inactive participant. 3) Number of fractures diagnosed in conjunction with the accidents under 2. 4) Number of femoral neck fractures.

To serve as controls 173 individuals were drawn from the population records of the city of Malmö who were identically distributed with regard to sex and age. The records of the 173 patients with arrhythmia and the 173 controls were searched in the same way and without knowledge of to which group the individual belonged.

RESULTS

The number of patients with one or more traumatic episodes leading to a roentgen examination was significantly increased—doubled—among the arrhythmia cases (Table II) ($0.01 > p > 0.001$, χ^2 test). Similarly, the number of fall accidents and similar accidents was significantly increased—more than doubled—in patients with episodic occult cardiac arrhythmia ($p < 0.001$). However, there was no significant difference between the two groups in the number of fractures resulting from all the trauma episodes. Consequently, the number of fractures in relation to the number of fall accidents was significantly less among the arrhythmia cases as compared with the controls ($0.01 > p > 0.001$). The increase in femoral neck fracture rate was not significant.

DISCUSSION

A coincidence of femoral neck fracture and episodic arrhythmia previously observed in our department could not be statistically substantiated in this study since the risk of this injury is still fairly low in the age groups included (4). It must be concluded that the risk of fall accidents increases during the years of occult arrhythmia episodes preceding the diagnosis. In a few instances the accidents may have been the predominant symptom that subsequently

led to the consultation and diagnosis, thereby inducing unknown but probably insignificant bias in the selection process. Finally, the fracture rate remained unchanged in spite of the increase in number of trauma incidents severe enough to require a roentgen examination. The additional requirement for low-energy fractures—a reduced bone mass (10)—may be absent in the arrhythmia cases of this series. The interaction of bone fractures and falls caused by Adams Stokes attacks, however, become important later in life.

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Episodic Cardiac Arrhythmia and Femoral Neck Fracture

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ABSTRACT Fifty-eight consecutive patients admitted to hospital for fracture of the upper end of the femur were examined by continuous ECG monitoring 22-24 hours. More than one-third of the patients had occult previously unknown episodic arrhythmias severe enough to cause dizzy spells and syncope. These patients had more often a history of dizziness and syncope and could less frequently than patients without serious arrhythmias return to their homes at the time of discharge despite uncomplicated fracture treatment.

Keywords: arrhythmia, ECG, fracture, femoral neck.

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Episodic cardiac arrhythmias may cause dizziness and fainting. In patients with such symptoms, serious episodic arrhythmias have been found by continuous ECG monitoring in incidences ranging from 20 to 74% (2, 5, 6, 7, 8, 11). Increased risk of falls is common in patients with episodic arrhythmia causing dizziness and syncope. In a series of patients with Adams-Stokes syndrome treated with pacing, no less than 13% had been admitted to hospital because of trauma prior to pacemaker implantation (3). This liability disappeared during pacemaker treatment. Transient cardiac arrhythmia is a elusive condition which may not be recognized a long time or ever.

Femoral neck fracture—fracture of the upper end of the femur—is related to aging (4) and impaired density of the bone and is regarded as one of the typical symptoms of bone fragility (9). However, it is generally agreed that a trauma in most instances is needed to cause a fracture, even if the trauma in itself would not cause injury to a subject with an intact bone quality.

The two conditions—fracture of the neck of the femur and occult cardiac arrhythmia—are both common in an aging population. The purpose of the present investigation was to study the possible role

of occult arrhythmias in patients with femoral neck fracture.

PATIENTS AND METHODS

Included in the study were 58 consecutive patients aged 51-91 (77±11): 12 men (aged 73±11) and 46 women (aged 78±11) admitted to the Orthopedic Department. Twenty-six of the fractures were trochanteric and 32 cervical. None of the patients had local bone destructions or other findings indicating a pathological fracture; one woman had a somewhat dubious impacted cervical fracture. The fractures were eventually treated with reduction and osteosynthesis, but were in all managed in traction for at least 24 hours of preoperative preparations. On admission, a detailed cardiac and neurological history was recorded in each case. A radiogram of the heart and lungs was performed in all patients in connection with the diagnosis of fracture.

Preoperatively, a continuous ECG monitoring covering 22-24 hours was performed using portable ECG tape recorders, type SRA/HRB 2 (Höglund Norden/Hellige). There was no change in the cardiac medication until the ECG recording was completed except for a few patients who received diuretics due to congestive heart failure. The ECG tapes were stored and analyzed after discharge using an automatic screening unit. Rhythms faster than 120 or slower than 40 beats/minute detected by single beat-to-beat interval triggered the screening unit to present the event as a written one-channel ECG on a paper strip. In addition, every 10th premature ventricular contraction (PVC) detected by QRS configuration criteria was presented. The further diagnostic procedure included a screening by specially trained laboratory technicians and finally by one of us (N. J. A.).

All signs of arrhythmia were recorded. Minor arrhythmias of types normally occurring in healthy subjects have been referred to as insignificant. Major arrhythmias with an inherent capacity of causing cerebral symptoms were considered significant. Such significant episodic arrhythmias were: Abundant PVCs—4 or more every 5th beat or more frequently—coupled PVCs and ventricular tachycardias defined as three consecutive PVCs at a rate of at least 100 beats/min; atrial tachycardias including fibrillation and flutter providing a ventricular rate of 120 beats/min or more and a duration of more than 1 sec; atrioventricular

Abbreviations: PVC=premature ventricular contraction; PAC=premature atrial contraction; SSS=sick sinus syndrome.

blocks of grades 2 or 3 and finally the sick sinus syndrome (SSS) fulfilling one or more of the following criteria: 1) Sinus bradyarrhythmias of 50 beats/min or less; 2) Regular sinus bradycardia of 45 beats/min or less when awake; 3) Sinoatrial block with more than one P wave missing; 4) Sinus arrests of 1.5 sec or more.

In addition to the monitored ECG, serial 12-lead routine ECGs were recorded and serial S-GOT and S-GPT determinations were made for three consecutive days after admission. This procedure revealed no case of definite or suspected acute cardiac disorder.

In addition to the detailed questionnaire used on admission, the record room of the Medical Department was searched for information on earlier complaints of dizzy spells and syncope as well as cardiac symptoms in the fracture patients. The record room of the Roentgen Department was also searched for information on trauma episodes for the last five years preceding the femoral neck fracture. The City of Malmö is served by one single hospital—the General Hospital—and the emergency room of the Medical Department is the only unit available for emergency consultations regarding neurological or cardiac symptoms. Thus, it is assumed that the overwhelming majority of such patients are registered in the record room of the department. Practically all trauma roentgen is also carried out in the General Hospital.

The fracture patients were compared with 103 individuals randomly selected from the population at risk: 51 men and 50 women with an average age of 71 years (range 47–92) who had been studied with regard to arrhythmias using the very same method of 22-hour continuous ECG monitoring. Out of these individuals, 26 had complaints of dizzy spells whereas 77 denied all forms of dizziness or syncope.

RESULTS

Patients with insignificant arrhythmias only

In 35 patients aged 75 ± 11 , no or only insignificant episodic arrhythmias were detected. One had chronic atrial fibrillation with an appropriate ventricular rate; 34 were in sinus rhythm. Fourteen patients had infrequent (i.e. less than every 10th beat) premature atrial contractions (PACs) and 8 had abundant or moderately frequent PACs (i.e. at least one PAC in 5 and 6–10 beats respectively). Two patients had short bursts of atrial tachycardias lasting for 8 sec or less. Eighteen patients had infrequent or moderately frequent PVCs.

Patients with significant episodic arrhythmias

In 23 patients aged 79 ± 10 , significant episodic arrhythmias were detected (Table I). Six patients had episodes of atrial tachycardias including fibrillation lasting longer than 8 sec with ventricular rates of 120 beats/min or more. Nine patients had abundant

Table I. Episodic arrhythmias in 77 asymptomatic controls and 58 patients with fracture of the neck of the femur

	Asymptomatic controls		Fracture cases
	N	%	N
SSS	3	4	2
Attacks of rapid supra-ventricular rhythms	2	3	6
High-frequency PVCs	4	5	9
ventricular bigeminy			3
Ventricular tachycardia			3
Atrioventricular block grade 2 or 3	9	12	23

$p < 0.001$ (χ^2 test)

PVCs or ventricular bigeminy and three had atrial tachycardias. Episodes of sinus bradycardia fulfilling the criteria used for SSS were present in two patients and transient atrioventricular block grade 2 or 3 was present in three patients.

Mortality and discharge from hospital

Five of the 23 patients with significant arrhythmias and four of 35 without died during hospitalization. This doubled mortality was however not statistically significant. Despite uncomplicated fracture healing, only 4 of the 18 surviving patients with significant arrhythmias had a general physical condition permitting return to their homes after discharge, whereas 15 of the 31 survivors without significant arrhythmias could return to their homes without permanent or transient need for medical care. Again, the difference was not significant. There were no signs of acute cardiac disease in any of the 58 patients—the subsequent departure in the cases was in most instances due to pulmonary embolism.

The ratio cervical/trochanteric fractures was 1/6 in the group without and 13/10 in the group with significant arrhythmias.

Previous illness and significant arrhythmias

Fifteen out of the 23 patients with and 12 out of 35 patients without significant episodic arrhythmias had visited the Department of Medicine because of dizziness and/or syncope or had reported the onset of symptoms on admission for fracture treatment.

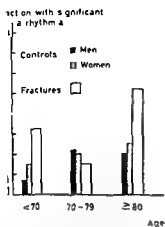


Fig. 1. Incidence of significant arrhythmia in fracture cases compared with all normals, symptomatic as well as asymptomatic.

At admission chest X ray showed cardiac enlargement in 5/23 of the arrhythmic cases and in 35 of those without significant arrhythmia. There is no significant difference in this aspect. However resting ECG showed definite previous myocardial infarction, ischaemic T wave inversions, arrhythmia in 7/23 arrhythmic patients and 1/34 patients without significant arrhythmia. This difference is statistically significant ($P < 0.01$).

There was no difference in the frequencies of earlier trauma episodes between fracture patients with and without significant arrhythmia.

Comparison with normals

Significant arrhythmia was approximately three times as common in femoral neck fracture cases as in the asymptomatic controls (Table 1). The arrhythmias in the fracture group were also more serious and long lasting than in the asymptomatic normals. However it must be taken into account that the age and sex distribution of the two groups is different. The 26 symptomatic normals who demonstrated significant arrhythmia in 12 cases could also be included in the comparison. The age and sex incidence of symptomatic arrhythmia is presented in Fig. 1 including all 103 normals. The incidence shows a tendency to increase with increasing age but does not differ significantly between the sexes. When comparing the femoral neck fracture cases with the normals, taking the age into account, there is still a significant difference in the

prevalence of arrhythmia among the youngest and the oldest in the two groups.

DISCUSSION

As pointed out above, serious episodic arrhythmias have been demonstrated in a high incidence in patients with dizziness and syncope. However only few asymptomatic individuals, even of advanced age, have serious episodic arrhythmias (2, 10). The findings of the present study indicate a significant increase in serious episodic arrhythmias, a possible contributory cause of fracture. This view is supported by the findings in 32 patients, 20 of whom had episodic arrhythmias treated for post apoplectic dementia in a psycho geriatric hospital in the City of Malmö. Not less than 11 had also sustained a fracture of the upper end of the femur. (1) Among 23 demented patients in the same hospital but without episodic arrhythmia, only 2 had had femoral neck fracture.

Fracture of the neck of the femur is a stressful condition, possibly capable of triggering arrhythmia. Arrhythmia then would be a result rather than a cause of the trauma. However, patients with significant arrhythmias had more often a history of dizziness than those without. Furthermore, since patients with significant arrhythmia had a markedly increased need of institutional care following the fracture episode, it might be argued that even if the trauma has triggered off the arrhythmia in some cases, this tendency might nevertheless be inherent with the patient and regardlessly resulting in a poor prognosis.

The mortality among our 58 fracture patients was about 15%, which is in agreement with the mortality figure in a previous study of the same population (4). Although the difference between the two groups is not significant, both the mortality and the ability of the patient to sustain without institutional care after discharge from hospital indicate a worse post fracture course for femoral neck fracture patients with serious arrhythmia, even if the age factor must be taken into consideration. The most serious prospect for these patients is probably the increased risk of continuing impairment of the cerebral function. The method of continuous ECG monitoring has proven its value in the diagnosis of occult episodic cardiac arrhythmia and will probably be a routine diagnostic tool in the future. Patients with fracture of the upper end of the femur appear to be a worthwhile target for such diagnostic efforts.

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Atrioventricular and Intraventricular Conduction in Familial Amyloidosis with Polyneuropathy

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ABSTRACT Atrioventricular and/or intraventricular conduction disturbances were found in 48 (68%) out of 71 patients with familial amyloidosis and polyneuropathy. Low voltage, often described in various forms of heart amyloidosis, was seen in one patient only. Myocardial infarction patterns, also reported in heart amyloidosis, were not present in our patients. The prevalence of conduction disturbances increased with the duration of the disease. In out of 47 patients from whom more than one ECG recording was available, a progression of conduction disturbances could be seen. Ten patients developed high degree conduction disturbances and required permanent pacemaker treatment.

Keywords: amyloidosis, ECG.
Acta Med Scand 208: 77, 1980.

During the last few decades several hereditary familial syndromes have been recognized which all have in common a systemic accumulation of amyloid and polyneuropathy (9). One type of familial amyloidosis with polyneuropathy (FAP) characterized by sensorimotor disturbances affecting the extremities more severely than the upper and widespread autonomic dysfunction was originally described in Portuguese patients (2). Kindreds with same disease have now also been reported from other countries: Japan (3), Sweden (1) and England (16).

In many of the various forms of primary amyloidosis described, heart affection is of major importance and these patients often succumb to heart failure (4, 7). The ECG findings most suggestive of cardiac amyloidosis are generally said to be low voltage, myocardial infarction patterns and to some degree rhythm and conduction disturbances (12). In previous reports on FAP of the type originally reported from Portugal, cardiac amyloid

deposits were common autopsy findings (6, 10, 11, 16). Angina pectoris and heart failure were uncommon among these patients but ECG signs of heart dysfunction were common (1, 15, 16).

The purpose of this report is to assess the occurrence of ECG abnormalities, especially QRS changes and conduction disturbances in Swedish patients with FAP. Special attention is paid to the natural history of conduction disturbances in this disease.

PATIENTS

The study concerns 71 patients, 46 men and 25 women, 66 of whom were admitted to the Department of Internal Medicine, University Hospital Umeå, and five to other hospitals in the Umeå region. The records of the latter five patients were available for our study. All patients had symptoms and signs of polyneuropathy and amyloidosis was confirmed in all during their life time by histological examination of biopsy material. No chronic inflammatory disease, myelomatosis or tumors were found in any of the patients. This study includes all known patients with verified FAP in the Umeå region with at least one available ECG recorded after the onset of symptoms.

The first symptoms had appeared at the age of 25-81 years (mean 55). Thirty-four patients are dead. They survived 4-21 years (mean 10) after the onset of symptoms. Thirty-seven patients are still alive and have been observed for 2-34 years (mean 9) after the onset of symptoms. ECGs were recorded at varying intervals. For 24 patients, only one recording was available; two or more

for 47. Digitalis had not been given to any of the patients with a high-degree conduction disturbance. Other drugs which can influence AV conduction—such as β blockers, quinidine or procainamide—were not given to any of our patients.

Abbreviations: FAP=familial amyloidosis with polyneuropathy; AF=atrial fibrillation; AV=atrioventricular; LV=intraventricular; LAH=left anterior hemiblock; LPH=left posterior hemiblock; LBBB=left bundle branch block; RBBB=right bundle branch block.

Table I Atrioventricular and intraventricular conduction defects in 71 patients with familial amyloidosis with polyneuropathy

Patients with LAH+RBBB are also included in the LAH and RBBB groups

	N	%
AV block	27	38
I	19	27
II	1	1
III	7	10
IV conduction defects	29	41
LAH	19	27
LBBB	7	10
RBBB	9	13
LAH+RBBB	6	9
AV block+IV conduction defect	9	13

METHODS

Biopsies for histological examinations were taken from skin, rectal mucosa or the sural nerve and the specimens were examined after Congo red staining in polarized light (13). A 12 lead ECG (I, II, III, aVR, aVL, aVF and six chest leads) with the conventional amplification (1 mV = 10 mm) and at a paper speed of 50 mm/sec was recorded using a direct writing ECG apparatus (Siemens Elema Ltd Solna, Sweden). The ECG evaluation included a classification of the ECG abnormalities according to the Minnesota code (14). The criteria of Castellanos and Lemberg (5) were used for left anterior and left posterior hemiblocks (LAH and LPH).

RESULTS

Abnormal ECGs were found in 62 (87%) of 71 patients. In nine patients the ECGs were normal 1–12 years after the onset of symptoms. Atrioventricular (AV) and/or intraventricular (IV) conduction defects occurred in 48 (67%) of the patients. The various conduction defects seen at the last investigation of each patient are given in Table I. Atrial fibrillation (AF) with or without IV conduction defects was present in 10 (14%) patients. Low voltage was found in one patient. No postinfarction changes were seen in this patient series. Nonspecific ECG changes such as occasional ventricular or supraventricular premature beats or repolarization abnormalities were not analyzed in detail in this study.

The prevalence of conduction disturbances increased with the duration of the disease and only three of the patients examined more than 10 years after the onset of symptoms had normal impulse

conduction. Table II shows the prevalence of conduction disturbances from ECGs recorded at various intervals.

Among 28 of the 47 patients from whom more than one ECG recording was available during the period of observation, the progression could be observed from normal impulse conduction to a form of AV or IV conduction disturbance or from a more low-degree to a more advanced conduction disturbance. Reversal of conduction disturbance was not seen. Ten of our patients required permanent pacemakers: seven because of AF, III, one because of AF with slow ventricular rate, left bundle branch block (LBBB) and fainting episodes, one because of AF, right bundle branch block (RBBB), LAH and fainting episodes, because of AV block, LBBB and Adams Stokes attacks.

Two case histories illustrate the prevalence and nature of the conduction disturbances.

Case 1

A female born in 1904 was admitted to the hospital for periods of varying length from 1962 to 1971. The family history revealed nothing of importance at the time of the first admission. FAP was diagnosed in two of her relatives. From 1958 onwards she had experienced increasing symptoms with paresthesias, numbness and muscular weakness and ataxia affecting the legs but extending a few years later to the upper extremities. From 1960 onwards she suffered from gastrointestinal disturbances with bouts of indigestion with periods of constipation.

The diagnosis of FAP was established in 1967 when amyloid deposits were found in biopsy material from the rectal mucosa. An ECG recorded on 10 Dec 1962 revealed AV block I with P-Q time 0.22 s. LAH. The heart volume was normal. The patient experienced periods with palpitations but had no heart symptoms. Fainting episodes occurred in the autumn of 1967 and AV block I with P-Q time 0.22 s, RBBB and LAH were found in Oct 1967. In Jan 1968 complete AV block with ventricular rate of 36/min was registered. A permanent right ventricular transvenous pacemaker was then inserted. From that time no further syncope episodes occurred and there were no cardiac complications.

The patient died suddenly from a cerebral thrombosis in Jan 1971. At autopsy the heart was found to be normal (weight 620 g). The coronary arteries showed no atherosclerosis. Abundant amyloid deposits were found in the myocardium. Amyloid deposits were also found in specimens from peripheral nerves, intestinal mucosa and the thyroid.

Case 2

A man born in 1905 into a family in which several members have FAP. He was in good health until 1961

the II Atrioventricular conduction defects and atrial fibrillation in patients with familial amyloidosis. The present study found different intervals after the onset of conduction system disease in the LAH and RBBB.

In more than one ECG was recorded in an inter- and intra-patient study. Findings in the last one are given. Findings at the recording prior to pacemaker implantation are given in parentheses. The patients with the II RBBB are also included in the LAH and RBBB groups.

Number of patients	Interval after onset of symptoms (y)					
	0-4		5-9		≥10	
	N	%	N	%	N	%
Normal ECG or non-specific ECG changes	5	50	9	76	3	19
Block	11	7	16	46	7	44
	8	16	13	37	4	5
	1	7	0	0	1	6
Conduction defects	2	4	3	9	7	13
LAH	1	4	11	31	4	25
RBBB	1	2	3	9	4	5
LAH RBBB	4	8	5	14	5	31
Block IV	2	4	3	9	2	13
Conduction defects	3	6	9	26	3	19
	6	12	3	9	3	19

In the present study, the patient had been on sufficient from progressive polyneuropathy for 10 years. From 1970 onwards he also had trouble with episodes of dizziness. The diagnosis was established in 1966 when amyloid deposits were found in biopsy material from the rectal mucosa. Progressive muscular atrophy and weight loss ensued but no history consistent with cardiac failure or angina pectoris and the patient had not experienced any fainting episodes. In September 1963 a normal ECG was recorded. In October 1966 the P-Q-T time still normal but RBBB was found. At the same time an X-ray showed normal heart volume and configuration. In March 1970 the last ECG recorded showed block I with P-Q-T time 32 ms RBBB and LAH. The patient died suddenly in February 1975. No autopsy was performed.

DISCUSSION

In a retrospective survey of the ECG findings in 100 patients with FAP we could not recognize a pattern often said to be more or less typical of many amyloidosis with heart affection that is a

high prevalence of low voltage and myocardial infarction patterns (4, 17).

The high prevalence of AV and IV conduction defects in our patients is consistent with the findings in a recent report on ECG changes in 190 Portuguese patients with FAP (8). One notable difference between our and the Portuguese patients is the lack of more high-degree conduction disturbances such as AV block III and bundle branch block among the Portuguese patients. However, the age difference between the Swedish and Portuguese patients makes it difficult to compare the two patient groups directly as does the lack of data in the Portuguese report concerning the duration of the disease.

In many of our patients for whom repeated ECG recordings were available we could observe a progression in the conduction defects. The case reports illustrate the stepwise interruption of impulse propagation which can be seen in these patients. The progressive conduction system disorder leads frequently to life-threatening disturbances and 10 of our 71 patients required pacemaker treatment.

Nothing is known about the pathophysiological background of the conduction disturbances in this disease. Amyloid infiltrations with special affinity to the conduction system might be one cause. However, if this is indeed the cause it is somewhat strange that there should be no LPH. Involvement of the autonomic nerves of the heart might be another contributory factor leading to the conduction disturbances. As yet there has been no histopathological examination of the conduction system in patients with FAP but such a study has now been initiated in our hospital.

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A Study of Stroke Patients Treated in a Non Intensive Stroke Unit or in General Medical Wards

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TRACT To study the representativity and outcome of patients admitted to a stroke unit (SU) 269) a comparison was made with all stroke patients treated in general medical wards (GMW) 225) in the same hospital during two years. There was no difference between the patient groups regarding sex, age, previous cardiovascular diseases or neurological deficit on admission. As expected, more neurological examinations were performed in the SU than in the GMW, where a diagnosis of ill-defined headache was very frequent. A higher frequency of lumbar puncture with CSF spectrophotometry would have increased considerably the number of specific diagnoses in the GMW. Acute and particularly secondary prophylactic treatment was more often given in the SU. There was no difference between the patient groups regarding mortality or length of hospital

stay. The Medical Department of Serafmerlasaretet in 1976 was to create a basis for scientific studies. Our aim was to evaluate diagnostic procedures in order to form an investigation program for non-selected patients of all ages with acute cerebrovascular disease (CVD). We also wanted to study different therapeutic measures during the acute phase of stroke as well as the effects of long-term secondary prophylactic treatment. As background to these studies, it was considered of value to compare stroke patients admitted to the SU with stroke patients treated in the general medical wards (GMW) during the same period.

The aims of the present study were to answer the following questions: 1) Were the stroke patients admitted to the SU representative of all patients with stroke treated in our hospital during the study period? 2) Did the diagnostic investigation program applied in the SU result in a different distribution of the diagnoses of CVD and more aggressive treatment than in the other wards? 3) Did the outcome during the hospital period differ between the two patient groups?

Keywords: acute cerebrovascular disease, prognosis.

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There have been several arguments for and against intensive care units or neurovascular units, mainly due to lack of effective specific medical treatment in the acute phase of cerebral infarction. Work in such units has concentrated on supervision of cardiovascular functions (7) and early and intensive treatment by medical and physiotherapeutic means (15). However, only a few authors have reported a decreased mortality or morbidity in these units (13). Most investigators have not been able to show any positive effects but have stressed the importance of the Stroke Unit (SU) as a basis for research and education (7, 8, 11, 14, 16, 17, 20). The reason for establishing a non-intensive SU at

PATIENTS AND METHODS

Serafmerlasaretet serves a population of about 1 000 inhabitants in Greater Stockholm and about 300 stroke patients are admitted annually. The Medical Department has 185 beds in eight medical wards. Except for the Coronary Care Unit there is no basic difference in patient care between the wards.

The study period comprised two years, Dec 1976-Nov 1978. Initially there were only five beds for women in the

Abbreviations: CVD = cerebrovascular disease, SU = stroke unit, GMW = general medical wards, TIA = transient ischaemic attacks, CSF = cerebrospinal fluid, ICD = international classification of diseases.

Table 1 Age and sex distribution and previous diseases registered in the admission form of patients treated in the SU or GMW

	SU (n = 769)	GMW (n = 725)
Men		
Mean age (y)	45 71	37 77
Women		
Mean age (y)	55 75	61 76
Previous illnesses (*)		
Stroke	19	17
TIA	11	9
Hypertension	47	39
Heart failure	31	76
Atrial fibrillation	18	70
Angina pectoris	14	11
Myocardial infarction	11	17
Diabetes mellitus	10	17
None of these	19	73

All differences were statistically non significant

SU. In April 1977 five beds for men were added. For this reason only women treated in the GMW were considered for comparison during the first four month period. After that and during the remaining study period all men and women with a diagnosis of acute CVD were included.

All patients with suspected acute CVD fulfilling the following admission criteria in the Casualty Department were admitted either to the SU if a bed was available or to a GMW: 1) Transient ischaemic attacks (TIA). Patients with one or more episodes of focal neurological deficit with a duration of less than 24 hours within the last month. Attacks of vertigo or syncope without focal neurological deficit were not included. 2) Progressive and manifest stroke. Patients with acute onset of focal neurological deficit during the previous week and without preceding trauma to the head.

In the Casualty Department a special record (*) based on a neurological score system was filled in by the examining doctor for all such patients. A high score ($\rightarrow 100$) meant a better prognosis for the hospital period (10). The history of present and past illnesses was also noted in these records. One of the authors (K. M.) checked the admission register at the Casualty Department every day to control all stroke patients admitted to the hospital. If necessary the patient was re-examined in the ward and the hospital record was consulted for missing data.

The Stroke Unit

The resources for general patient care in the SU did not differ from those in the GMW. There were no facilities for intensive care in the unit. A preplanned investigation program including lumbar puncture with spectrophotometry, skull X-ray with echoencephalography

and brain scan was adhered to. Strict criteria for admission and treatment were followed as described elsewhere (3, 4).

All categories of personnel have been specialized at weekly conferences and an early active mobilization and rehabilitation planning has been used. A close co-operation between all categories of personnel has also been developed.

Minimum diagnostic requirements

(A) Fulfilment of admission criteria (B) 1) Cerebral haemorrhage according to international classification of diseases (ICD) (74) (ICD 431). Macroscopic or rhagmic cerebrospinal fluid (CSF) or bleeding on spectrophotometry. 2) Cerebral thrombosis or cerebral arterial occlusion (ICD 432, 433). Absence of bleeding pattern at spectrophotometry in accordance with Cerebral embolism (ICD 434). Sudden onset of symptoms in patients with atrial fibrillation, valvular disease or atherothrombotic changes in the relevant artery. Findings at spectrophotometry in accordance with ischaemic lesions. 4) TIA (ICD 435). Focal neurological deficit with a duration of less than 24 hours. 5) but ill-defined CVD (ICD 436). Patients with CVD not fulfilling the minimum requirements for a specific nosology.

General medical wards

The principles of investigation and management of patients differed between medical wards according to routines of the consultant physician. A discharge summary is written for all patients at the time of discharge. The hospital records and summaries of all patients with a diagnosis of acute CVD fulfilling the admission criteria were studied with regard to present and past medical examination, results of laboratory and X-ray material on treatment and progress during hospital diagnosis and discharge. If our neurologist had consulted, his report was studied and so were all reports. For the purpose of comparing the GMW with those treated in the SU the final diagnoses and discharge summaries were reconsidered and when necessary changed according to the diagnostic criteria above.

Statistics

The χ^2 test was used for testing the significance of differences of proportions. Two-sample t test was used for differences between means. Degrees of significance tested at 5% and 0.1% levels.

RESULTS

General characteristics of the patients (Table I)

During the study period a final diagnosis of CVD was established at discharge in 769 patients, age 73 years, range 50-97 in the SU and 72 in the

Table II Diagnoses in the discharge summaries of patients treated in the GMW and the final diagnoses according to the diagnostic criteria of the SU

Diagnoses in the discharge summaries	Final diagnoses				
	Cerebral haemorrhage	Cerebral thrombosis	Cerebral embolism	TIA	Acute ill-defined CVD
Cerebral haemorrhage	16	3			4
Cerebral thrombosis		17	4		7
Cerebral embolism		4	17		8
Acute ill-defined CVD	7	3	16	2	6
Total	23	27	37	31	71

patients (mean age 74 years, range 41-100) in the GMW. Somewhat more men than women were admitted to the SU but the difference was not statistically significant. The mean neurological score on admission was 61 in both groups. The prevalence of important previous diseases did not differ between the two groups.

Diagnostic investigations

Lumbar puncture with CSF spectrophotometry was performed in 94% of cases in the SU as compared to 50% in the GMW ($p < 0.001$). Skull X-ray and computerized tomography were performed in 94% and 83% in the SU compared to 35% and 50% respectively in the GMW ($p < 0.001$). Archival angiography was performed as often in the GMW (50%) as in the SU (11%). Autopsies were performed in 45 (97%) of 49 deceased in the SU and in 73% of 45 in the GMW.

Distribution of diagnoses

After reconsideration the diagnoses were changed in 84 patients (37%) treated in the GMW (Table II). The final distribution of diagnoses is presented in Table III. Since less investigations were performed in the GMW the ill-defined group was considerably larger there than in the SU. Cerebral thrombosis and cerebral embolism were significantly more common diagnoses in the SU while TIA was more frequent in the GMW.

Treatment and secondary prophylaxis

Of the SU patients with repeated TIA or progressive stroke received heparin compared to two patients with TIA and one with progressive stroke in the GMW. Of the SU patients with cerebral infarction 46 were included in a double-blind study with

continuous intravenous theophylline (5). Of the patients with non-haemorrhagic diagnoses and discharged alive 72% in the SU and 35% in the GMW received acetylsalicylic acid and/or dipyridamol as prophylactic treatment. Anticoagulant treatment (warfarin) was instituted in 13% of the SU patients and in 17% of the GMW patients. Carotid endarterectomies were performed in 8 patients in the SU and 7 in the GMW.

Short-term outcome

There was no difference in hospital mortality or length of hospital stay (71 days in the SU and 70 days in the GMW) between the two patient groups. The proportions of patients sent home and to rehabilitation hospitals were also similar (Table IV).

DISCUSSION

The criteria for admission to the SU have previously been shown to be valid (sensitivity 86%, specificity 99%) for identification of patients with suspected CVD admitted to hospital (1). The pa-

Table III Percentage distribution of final diagnoses and mortality (in parentheses) in the SU and GMW

	SU (n = 269)	GMW (n = 25)	p
Cerebral haemorrhage	8 (50)	11 (61)	NS
Cerebral thrombosis	58 (12)	25 (10)	<0.001
Cerebral embolism	24 (27)	16 (2)	<0.05
TIA	8 (0)	14 (0)	<0.05
Acute ill-defined CVD	7 (40)	34 (19)	<0.001

p values indicate differences in diagnoses, differences in mortality were not significant.

Table IV. Mortality and discharge after the hospital period (%)

	SU (n = 713)	GMW (n = 225)
Hospital mortality	18	16
Discharged		
Home	44	48
To rehabilitation hospital	36	33
To other clinics	7	1

All differences were statistically insignificant.

tion with suspected stroke who came to the Casualty Department were not to be admitted to the SU or to the GMW without any selection. Nevertheless, some selection may occur in spite of such an intention, e.g. more serious ill as well as younger patients are preferably transferred to the specialized unit (20). There was, however, no difference between the two patient groups as regards their age or degree of neurological deficit on admission nor the prevalence of previous diseases. The aim to bring about a non-selected group of patients with stroke admitted to the SU was achieved.

Lumbar puncture with CSF spectrophotometry, skull X-ray, echocardiography and brain scan were included in the investigation program in the SL and were consequently performed more often there than in the GMW. Arch angiographies were made as often in the GMW as in the SL and were performed in the SL at the final investigation in patients considered for carotid endarterectomy (patients with TIA or a brain infarction with minor neurological deficits). In the GMW, arch angiography was not always a preoperative investigation but a decision, e.g. it had been performed in three patients more than 5 years and hardly considered for carotid endarterectomy and in another four without prior CSF analysis. Criteria for angiography should be used to avoid unnecessary risks and the investigation is mainly to be regarded as preoperative.

A higher autopsy rate in the SL than in the GMW and a longer clinical investigation secured a high reliability of the diagnoses in the SL and a distribution of diagnoses in good agreement with other authors (25). Compared to this, more than one third of the GMW patients had a final diagnosis of ill-defined CVD before application of the diagnostic criteria of the SL, more than half (51%) of the patients had this diagnosis. These findings indicate

that a discussion about criteria for diagnosis of different types of stroke should be of practical use. It is possible that the high age of the patients and the lack of known efficient medical treatment of acute stroke influence negatively the speed of differentiation between cerebral infarction and haemorrhage. A more consequent use of analysis would have diminished considerably ill-defined stroke group in the GMW.

There was no difference in total hospital mortality or length of hospital stay between the two groups. Although early rehabilitation and collaboration between different categories of the hospital were stressed in the SU, it must be remembered that our SU is not an intensive care unit. The present finding of no difference in hospital mortality between the SU and the GMW was expected. Some authors have reported that in cerebral stroke patients does not decrease hospital mortality (14, 16, 17, 20). Others have shown improvement regarding the stroke patients' functional capacity for early intensive mobilization at habilitation (12, 13, 15). It could not be evaluated whether other wish effects were achieved in our study. Standardized assessments of the functional capacity were not made consequently the GMW during the study period.

Acute medical treatment with heparin, thrombolytic stroke was given somewhat more frequently in the SU than in the GMW. The thrombolysis was carried out in some SU patients with brain infarction (4) was more intensive and probably have influenced the outcome of the SU patients.

Secondary prophylactic treatment with aspirin and/or carotid endarterectomy have generally used as a prophylactic measure against embolism and TIA (9, 18, 19, 22) and treatment with aspirin and has been shown to be of value in patients with TIA (6). Platelet aggregation with aspirin and/or carotid endarterectomy has been given to 85% of the patients with a non-haemorrhagic lesion in the SL and 47% of the patients in the GMW. However, one third of the latter had a diagnosis of ill-defined CVD, e.g. anticoagulants had been given to some without prior further puncture. Although beneficial effects of these vascular prophylactic measures still remain to be definitely proved, it is obvious that such treatment should not be given before a specific diagnosis of CVD has been established.

f computerized tomography is not available
bar puncture with CSF spectrophotometry
uld be used for differentiation between haemor
gic and ischaemic cerebral lesions (21)

The following conclusions could be drawn from
present study 1) An unselected and representa
e group of patients with acute stroke was admit
l to the SU 2) A higher frequency of ill defined
D was seen in the GMW than in the SU A more
requent use of spectrophotometry of CSF in
ients treated in the GMW would have increased
nsiderably the accuracy of diagnosis The better
ality of diagnosis in the SU did result in a more
quent and correct use of secondary drug
phylaxis 3) Short term outcome did not differ
ween patients treated in the SU and in the
GW

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Vitamin D Deficiency in Welfare Institutions for the Aged

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STRACT Serum concentrations of 25 OH D in a group of 47 elderly people (70-94 years old) living in homes for the aged were lower than those in a matched control group living in their homes. No differences between the groups were noted in serum ionized calcium, alkaline phosphatase, inorganic phosphate, magnesium or parathyroid hormone. The serum concentration of 25 OH D may be due to outdoor activities and/or a smaller dietary vitamin D intake in the institutionalized group. The importance of preserving an adequate vitamin D status in geriatric patients is emphasized.

Keywords: 25 hydroxyvitamin D, age, institution.
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Vitamin D deficiency is reported to be common in the elderly, especially in housebound and hospitalized people. Deficient exposure to daylight is considered to be the main cause of vitamin D deficiency in the elderly (4, 14), with malnutrition (1), malabsorption (2) and certain drugs (8) as possible contributory factors.

Vitamin D deficiency may conveniently be detected by measurements of the vitamin D metabolite 25 OH D in serum (15). Vir and Love (16) thus found lower serum 25 OH D values in institutionalized elderly people than in outpatients of the same age, which is of considerable interest with respect to the care of geriatric patients.

The aim of this study was to investigate if vitamin deficiency exists in ambulant people living in residential institutions for the aged. The serum concentrations of 25 OH D, ionized calcium, total calcium, alkaline phosphatase, phosphate, magnesium and parathyroid hormone were determined

in three homes for the aged: Bedridden people and people with gastrointestinal disease, such as partial gastric resection, or who were on drugs known to affect vitamin D status, e.g. barbiturates and phenytoin, were excluded. Minor disabilities such as arthrosis, vertigo, parkinsonism and cardiac compensation were accepted as well as intake of frusemide, digoxin, diazepam, paracetamol, hydrochlorazone, propranolol and aluminium hydroxide. All the subjects were ambulant.

A reference group, matched by age, sex and general health status, was selected from the Geriatric Out Patient Department. All these subjects were living in their own homes.

The study group and the control group were investigated at the same season (April and May) in order to eliminate seasonal variations in vitamin D metabolism.

METHODS

Serum 25 OH D was measured by competitive protein binding assay according to Bouillon et al. (3) with the modification that gelatin was used instead of lipoprotein in order to diminish adsorption effects. The coefficient of variation as determined in intra assay was 11% at 20 nmol/l ($n=10$) and 12% at 50 nmol/l ($n=14$). Corresponding values as determined in interassay were 20% at 20 nmol/l ($n=16$) and 15% at 50 nmol/l ($n=26$). Lower limit of sensitivity was 4 nmol/l. Inorganic phosphate and alkaline phosphatase concentrations in serum were determined with continuous flow system on a SMAC instrument (Technicon Instruments, Tarrytown, NY). Serum ionized calcium was analyzed with an ion selective flow through system (Orion SS 20, Orion Research, Cambridge, Mass.) (10). Serum magnesium concentration was determined by atomic absorption spectrometry and serum parathyroid hormone concentration was analyzed according to Kleerekoper et al. (9). Time spent outdoors and the dietary vitamin D intake during the last two days were estimated by direct interview with the subject (12). Matched groups were interviewed on the same day.

RESULTS

Mean values for vitamin D intake and time spent outdoors were somewhat lower in the study group than in the reference group, but the statistical sig-

The study group comprising 24 women and 23 men (mean age 84, range 70-90 years) was randomly selected from

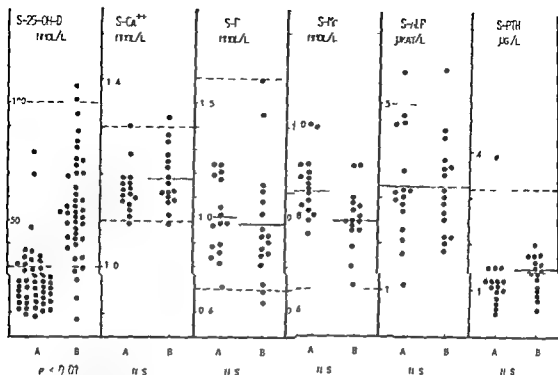


Fig 1 Biochemical findings in elderly people living in homes for the aged (A) and in people living in their own homes (B) — Sample mean — normal range

nificance was difficult to validate as the available data were too imprecise.

Determinations of 25 OH D levels revealed that the study group had lower values of this vitamin D metabolite than the control group ($p < 0.01$) (Fig 1).

Certain other laboratory tests for variables known to be influenced by vitamin D deficiency: serum ionized calcium, inorganic phosphate, magnesium, alkaline phosphatase and parathyroid hormone were also carried out in a subgroup of 16 institutionalized subjects and 16 controls (Fig 1). No significant differences in these variables were detected between institutionalized and non-institutionalized subjects. Two of the 16 institutionalized subjects in this subgroup had had a fracture during the last 3 years, while three fractures had occurred among the 16 controls.

DISCUSSION

This study is a confirmation of the findings of Vir and Love (16). The subjects with low and normal S-25 OH D did not differ as regards alkaline phosphatase, inorganic phosphate, ionized calcium or

parathyroid hormone concentrations. Measurements of S-25 OH D are accordingly valuable in revealing subclinical vitamin D deficiency. Osteomalacia, however, can only be demonstrated by histopathological examination, which was not performed in this study. It should be noted in this context that bone fracture is a late symptom of vitamin D deficiency, whereas muscular weakness, fatigue and bone pain are more common but are often overlooked (13). As 16 of 47 subjects in the study group had S-25 OH D values of less than 20 nmol/L, a level below which the frequency of osteomalacia is reported to be increased (5), vitamin D supplements might be of value. This should be feasible. MacLennan and Hamilton (11) observed that peroral supplements did increase S-25 OH D in elderly people and Coreless *et al* (7) found increased S-25 OH D concentrations in elderly people after exposure to UV light.

There are several possible reasons for the low serum concentrations of 25 OH D observed in the study. Attempts to assess the duration of outdoor stay indicated that the institutionalized people had less outdoor activities than the control group.

interview technique probably gave too imprecise results for a definite conclusion. Dietary differences between the two groups may also be responsible for the low 25 OH D level. Malabsorption of vitamin D, reduced hepatic 25 hydroxylation or increased vitamin D elimination may be additional contributory factors.

Thus we found low 25 OH D in a group of ambulant elderly people living in homes for the aged. The biological significance of this observation needs to be further elucidated.

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Serum Reverse- T_3 Determinations in the Laboratory Diagnosis of Hyperthyroidism

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ABSTRACT The relative discriminatory value of the determination of the serum reverse- T_3 levels for the laboratory diagnosis of hyperthyroidism was investigated in 47 patients with clinical signs or symptoms of thyroidism. The results were compared with those from the determination of the total serum levels of T_3 and T_4 prior and after correction for the binding proteins. Twenty-three of the patients had normal thyroid function and 24 had hyperthyroidism. The determination of the total serum T_3 level was superior to the determination of the total serum T_4 and the reverse T_3 levels even subsequent to correction for binding proteins.

Key words: reverse T_3 , thyroxine, triiodothyronine, thyroid function, hyperthyroidism.

Acta Med Scand 208 91 1980

A recent development of specific radioimmunoassays for determination of reverse T_3 now commercially available, there has been considerable interest in the role of this compound in various conditions (2, 3, 11). The relation between T_3 and reverse T_3 levels have been of special interest. It is not yet known if the reciprocal alterations in T_3 and reverse T_3 levels seen in different thyroidal diseases represent homeostatic adjustments at energy conservation by preferential T_4 adaptation to reverse T_3 rather than to T_3 or are caused by altered metabolic clearance. The different results have recently been summarized (1). The clinical value of reverse T_3 determination for laboratory diagnosis of hypo and hyperthyroidism is not fully known. The discriminatory power of reverse T_3 determination in patients with possible hypothyroidism seems to be inferior to the determination of TSH. The reason is that a normal T_3 level excludes primary hypothyroidism while as much as 40% of hypothyroid patients have been

reported to have reverse T_3 levels in the normal range (9). Reverse T_3 determinations in the laboratory diagnosis of hyperthyroidism have shown elevated levels when compared to normals (1, 4, 7, 9). Some of the hyperthyroid patients were found to have reverse T_3 levels in the normal range. However, the discriminatory power of reverse T_3 determination for this diagnosis compared to the determination of T_3 or T_4 levels before and after correction for the hormone binding proteins is unclear.

The aim of the present investigation was to study the relative clinical value of reverse T_3 determinations in patients with possible hyperthyroidism.

STUDY POPULATION AND METHODS

In a recent investigation the discriminatory value of T_3 and T_4 determinations before and after correction for binding proteins and other functional tests were elucidated in 50 patients with clinical signs or symptoms of hyperthyroidism. Each patient underwent a thorough clinical and laboratory investigation in order to establish the state of the thyroid function. The results showed that 26 of the 50 patients had normal thyroid function and 24 had hyperthyroidism. The study population, laboratory methods and results have been reported (8). The reverse T_3 levels have now been analyzed in these patients. Due to the lack of serum, three of the normals had to be excluded. Reverse T_3 was determined by a commercially available kit (Hypolab SD, Cönsins, Switzerland). The reference interval (\pm SD) was 0.34 ± 0.10 nmol/l. The T_3 uptake (T_3U) test was performed using Sephadex as adsorbent (12). The free reverse T_3 index was calculated according to the formula for the free T_4 index (5): $(\text{reverse } T_3) \times (T_3U)/100$.

RESULTS

The mean levels and derived parameters of reverse T_3 , T_3 and T_4 in the normal and hyperthyroid group are shown in Table I. It can be seen that the mean levels in the normal and hyperthyroid group are clearly separated.

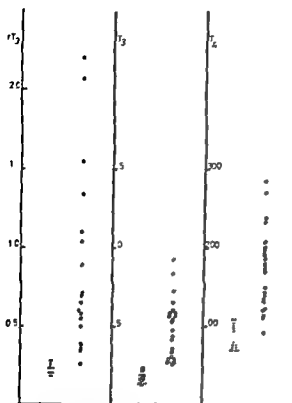


Fig. 1 Serum levels of reverse T_3 (rT_3), T_3 and T_4 in the normal (●) and hyperthyroid patients (○)

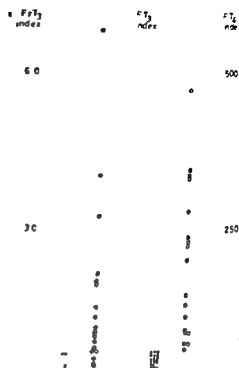


Fig. 2 Derived parameters of rT_3 , T_3 and T_4 in normal and hyperthyroid patients. Symbols as in Fig. 1

The levels of reverse T_3 , T_3 and T_4 of the individual patients before and after correction for the influence of the binding proteins are shown in Figs 1 and 2. It can be seen that the determination of T_3 is superior to both the determination of T_4 and reverse T_3 for the laboratory discrimination between eu- and hyperthyroidism. Correction for the binding proteins slightly improved the separation of the two groups.

The overlap in reverse T_3 levels between the normal and the hyperthyroid group indicates that the determination of reverse T_3 is not a suitable test as a discriminant between the two groups. Similar conclusion can be made for the T_4 determination.

DISCUSSION

Even if increased levels of reverse T_3 in hyperthyroidism have been described, several objections to its clinical usefulness may be raised from the theoretical point of view. One objection is that the monodeiodination of T_4 to reverse T_3 is increased

during non-thyroidal conditions like hepatic cirrhosis (4), acute febrile illness (10), malnutrition and other systematic disorders with simultaneous decreased T_3 levels. Despite euthyroidism, elevated reverse T_3 levels may be seen in normal subjects.

However, the same objection regarding its diagnostic usefulness could be raised for T_3 determinations as T_3 levels are simultaneously decreased under similar conditions. Clinically, the objection may not be of any major importance as the discriminatory power of T_3 determinations is well established for the routine laboratory diagnosis of hyperthyroidism (14).

Other objections have been based upon the fact that reverse T_3 is derived from extrathyroidal deiodination and that only a minute amount is produced by the thyroid (4, 6). Thus, the concentration does not necessarily reflect the function of the thyroid gland (13). Furthermore, reverse T_3 is a biologically inactive compound and may therefore be rather inappropriate to determine for diagnosis of a hypermetabolic condition (6).

Table 1 rT_3 , T_3 , T_4 (nmol/l) and derived indices in euthyroid and hyperthyroid patients

	Euthyroid	Hyperthyroid
rT_3	23 11.26±0.18	24 0.91±0.76
rT_3 index	19 0.28±0.19	19 1.71±1.59
T_3	23 1.67±0.41	24 4.64±1.94
T_3 index	19 1.79±0.29	19 7.78±4.91
T_4	23 88.0±23.0	24 173.0±50.0
T_4 index	19 94.8±18.8	19 281.5±128.8

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Report on a Patient with Watery Diarrhoea Syndrome Caused by a Pancreatic Tumour Containing Neurotensin, Enkephalin and Calcitonin

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ABSTRACT A patient with watery diarrhoea syndrome (WDS) is described. A pancreatic tumour was found containing many cells with immunoreactivity for neurotensin, enkephalin and calcitonin, a few with immunoreactivity to vasoactive intestinal peptide (VIP). High levels of calcitonin, neurotensin, VIP and pancreatic polypeptide (PP) were present in plasma as measured by radioimmunoassay. After removal of the tumour, the plasma levels of the first three peptides returned to normal and the WDS disappeared. On the other hand, plasma levels of PP did not change. No specific symptoms could be attributed to the new spectrum of peptides found in the tumour. This is the first report of a pancreatic tumour containing high levels of neurotensin.

Keywords: watery diarrhoea, VIP, neurotensin, enkephalin, calcitonin, PP, pancreatic tumour.
Acta Med Scand 208: 95, 1980.

The watery diarrhoea syndrome (WDS) first described in 1958 (49) is characterized by intractable watery diarrhoea, hypokalaemia, impaired glucose tolerance and an islet cell tumour of non- β -cell type. It has been suggested that the syndrome is caused by tumours producing vasoactive intestinal peptide (VIP) (4, 40). However, the disease has also been described in patients with normal VIP values but increased levels of calcitonin, gastrin, glucagon, serotonin (41), prostaglandin (28) and pancreatic polypeptide (PP) (31). Since many of the tumours in patients with WDS secreted more than one peptide, it has been difficult to define the one responsible for the specific symptoms of this syndrome.

A patient with WDS and pancreatic tumour containing neurotensin, enkephalin and calcitonin is described. The clinical and laboratory findings with this new spectrum of hormone overproduction are reported.

PATIENT REPORT AND RESULTS OF SPECIAL STUDIES

The patient, a 65-year-old man, had always enjoyed good health apart from a thyroidectomy because of toxic colloid goiter 20 years ago. He was receiving substitution therapy with 75 mg of a desiccated thyroid preparation daily. His mother also suffered from goiter but otherwise there were no endocrine diseases in the family history.

During the last 15 years before admission the patient had observed periods of gradually increasing watery diarrhoea with up to 15 urgent calls to stool per day and faecal volumes exceeding one litre per day. No mucus or blood was observed in the stool. During these attacks he often experienced minute-long flushes localized to head and trunk but no nausea or vomiting. In the last six months he had lost 15 kg in weight and suffered from muscular weakness. There were no symptoms of gastritis, respiratory depression, kidney failure or polyuria, nor any mental changes or general analgesia.

All haematological indices and liver function tests were normal. Fasting blood glucose was 11.5, potassium 3.0, sodium 136, calcium 3.3, phosphate 1.2 mmol/l. Creatinine was normal. Serum thyroxine and triiodothyronine two months after withdrawal of thyroid substitution were normal. Administration of 200 μ g TRH was however not followed by any increase in TSH.

Abbreviations: WDS = watery diarrhoea syndrome; VIP = vasoactive intestinal peptide; PP = pancreatic polypeptide.

Table 1 Polypeptide content (pmol/l) in plasma and presence of immunoreactive cells in the tumour
 NTLI = neurotensin-like immunoreactivity PPLI = PP-like immunoreactivity VIPLI = VIP-like immunoreactivity

Polypeptide	Normal range in plasma	Preoperative plasma	Peroperative tumour	Postoperative plasma
Calcitonin	120-290	5000	+	160
NTLI	<600	3200	+	24.5
PPLI	<400	1400	+	1300
VIPLI	<20	250	-	3.5

Calcified tissue was demonstrated on X ray in the right lobe of the thyroid gland. Scintigraphy showed a normal ^{125}I uptake and distribution. A fine needle biopsy yielded normal tissue. Urinary excretion of norepinephrine, epinephrine, 4-hydroxy-3-metoxymandelic acid and 5-hydroxyindolic acetate before and during diarrhoea attacks were normal.

Plasma growth hormone, prolactin, glucagon, LH, FSH, insulin and testosterone were all normal. Serum gastrin was within the normal range. The secretion of gastric acid was low before and after pentagastrin stimulation (6 $\mu\text{g/kg}$ b.wt.). Determination of calcitonin-like, neurotensin-like, PP-like and VIP-like immunoreactivity showed markedly increased values in plasma (Table 1).

The patient's mild diabetes was controlled by diet alone. The hypercalcaemia normalized upon rehydration. The hypokalaemia was corrected by 4.5 g KCl p.o. daily. The watery diarrhoea responded only moderately to opiates but no effect of indomethacin 100 mg/day (an inhibitor of prostaglandin synthesis) was observed. A computerized tomogram revealed a tumour in the caudal part of the pancreas despite a negative angiography.

At surgery a solid encapsulated tumour (5x5x4 cm) was resected with a distal pancreatectomy. No metastases were found at operation. The diarrhoea, flushing, muscular weakness and hypokalaemia subsided. However the patient developed hypertension postoperatively (210/110 mmHg). He was back at work after eight weeks and soon regained normal body weight.

Fasting blood glucose levels were normal on a regular diet but the i.v. glucose tolerance test showed a decreased K value (0.80). The unresponsiveness to TRH remained unaltered. Plasma calcitonin, neurotensin and VIP-like immunoreactivity were normal one month and six months after the operation but the PP values remained raised (Table 1).

METHODS

Radioimmunoassays

All blood samples for peptide and hormone assays were collected in chilled glass test tubes. Trasylol® (Bayer, Leverkusen) was added (10% of volume) to inhibit proteolysis. The samples were centrifuged at 4°C and the plasma was frozen. Plasma for PP and VIP assays was freeze-dried and stored at -70°C. VIP was analyzed as

described previously (20). PP was determined using a phase radioimmunoassay procedure (12). PP antisera as well as pure human PP for standards were gifts from Chance Ebb Lilly, Minneapolis.

The methods used for radioimmunoassay of neurotensin will be presented in detail elsewhere and only a brief description of the method is given here. The antiserum (0-7701) was raised in rabbits and reacted with neurotensin (NT), NT (1-12) (Gln¹²) and (Gln¹³) NT (1-11) but with NT (4-13) or smaller C-terminal fragments of it. It showed no cross-reactivity with gastrin, bombesin, polypeptide porcine VIP, porcine secretin, cholecystokinin 33, cholecystokinin 34, cholecystokinin 39, creatine glucagon, species substance P, insulin, somatostatin, bovine pancreatic polypeptide, bombesin, gastrin 17, gastrin 34 or with trypsin, trypsin 34 which presumably contains the N-terminal decapeptide. Neurotensin-like immunoreactivity in plasma from the patient eluted from a Sephadex column as a single peak. Calcitonin, human GH, glucagon, insulin, testosterone, FSH, LH and P were analyzed by radioimmunoassay.

Histopathological and immunohistochemical analyses

Thin tumour slices were fixed in 10% buffered formalin for 12 or 24 hours. The fixed specimens were rinsed overnight in a phosphate buffer with 5% Cryostat sections, 10 μm thick, were processed according to the indirect immunofluorescence technique of (12). Other tissue samples were embedded in paraffin, dehydrated and cleared in xylene. Paraffin sections, 5 μm thick, were stained with haematoxylin-eosin, processed for argentaffin (43) with argyrophil technique (25-43). Sections were also stained with the peroxidase technique (46).

The following antisera were used on the cryostat sections for somatostatin (no. R141 C) (16), gastrin (no. 38), VIP (no. 5603 T) (17), substance P (no. 125) neurotensin (8-29), serotonin (45) and methionine-enkephalin (no. 336) (42). Two insulin antisera (a gift from Wide) glucagon (50) and bovine IGF (24) were used for paraffinized sections.

The calcitonin antibody was raised in rabbits after conjugation of synthetic human calcitonin to bovine albumin. In both immunohistochemical techniques the sections were incubated with antisera at +4°C for 24 hours according to Goldman (21).

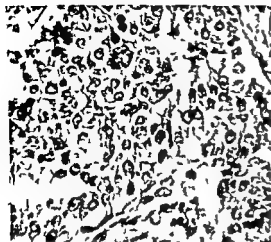


Fig 1 Tumour cells with mainly solid arrangement and slight polymorphism. Many tumour cells contain cytoplasmic vacuoles often located to the capillary pole of the cell. HE $\times 350$.



Fig 2 (A) Immunofluorescence micrographs of the pancreatic tumour. Many cells exhibiting strong neurotensin immunoreactivity can be seen. (B) A subpopulation of enkephalin immunoreactive cells in the adjacent section. Bars indicate 50 μm .

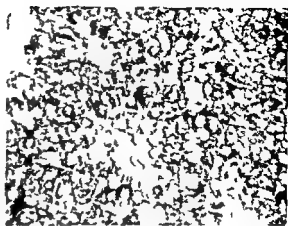


Fig 3 Section from the tumour stained with calcitonin antiserum (PAP technique).

Histology and immunohistochemistry

The tumour cells mainly showed a solid arrangement with multifocal necroses. In most areas the stroma was fairly richly vascularized but contained sparse strands of connective tissue. Most of the tumour cells contained a moderate amount of weakly eosinophilic stained cytoplasm. In many the cytoplasm contained vacuoles of varying size often concentrated to the capillary of the cell. Most nuclei showed only a slight pleomorphism but some giant nuclei were seen. In some areas the nuclei contained prominent nucleoli. Very few mitoses were observed (Fig 1).

Almost all tumour cells displayed argyrophilia with the Grimelius stain indicating their endocrine nature. With the Sevier Munger technique the staining reaction varied in different parts of the tumour. In some areas the majority of cells showed argyrophil reaction while in others only few cells were stained. No silver positive reaction was observed with the Masson and the Hellerstrom-Hellman techniques indicating absence of serotonin and somatostatin respectively.

The tumour contained cells with immunoreactivity to neurotensin, methionine-enkephalin and calcitonin. A few PP immunoreactive cells were also seen. In some areas the majority of cells showed neurotensin-like immunoreactivity (Fig 2). In the same areas a minority of cells were enkephalin immunoreactive but it was not possible to determine if the same cells showed immunoreactivity with both antisera. Groups of cells showing calcitonin immunoreactivity occurred in other areas of the tumour where no neurotensin or enkephalin reactivity could be demonstrated (Fig 3).

None of the other antisera tested including VIP antiserum revealed any immunoreactive cells. Furthermore there were areas in the tumour with argyphilic cells which did not show any immunoreactivity at all.

DISCUSSION

No single etiological agent which could be responsible for the WDS has been established so far. VIP

Table II Normal localization pharmacophysiological effects and observed clinical symptoms of peptides found in the tumour and serum of the patient

Peptide	Localization	Ref no	Effects		Ref no
			Pharmacophysiological	Clinical	
Calcitonin	C-cells in thyroid calcitonin cells in pancreatic endocrine tumours	37	Ca ²⁺ ↓ Mg ²⁺ ↑ stimulate small intestinal juice production	Diarrhoea*	13 22
Enkephalin	Many areas of the brain except the pituitary Highest conc in antrum duodenum pancreas ileum gallbladder and lesser amounts in colon	36	Opiate like central nervous system effects respiratory depression in creased gastric motility Central vasomotor depression release of ADH prolactin	Euphoria analgesia flush* constipation emesis hypotonia*	1 14* 37 47
Neurotensin	Ileal mucosa less in the jejunum traces in the stomach duodenum and colon Hypothalamus and other areas of the brain	10 40	Inhibition of intestinal motility vasodil in small intestine Vasoconstriction in adipose tissue Inhibition of gastric acid secretion Stimulation of glucagon release Inhibition of insulin release enhancement of glycogenolysis Role in postprandial processes	Diabetes inhibited gastric acid secretion* hypotonia*	2 3 9 11 33
PP	At the periphery of pancreatic islets in pancreatic acinar tissue few cells in the duodenum	20	Stimulates basal acid secretion inhibits pentagastrin stimulated Enhances secretin-induced pancreatic secretion (low dose) inhibits stimulated pancreatic secretion (high dose)	Diarrhoea* inhibited gastric acid secretion	19 31
VIP	Widely distributed throughout the gastrointestinal tract from lower oesophagus duodenum to the colon and rectum as well as in the pancreas Nervous tissues e.g. as mesent ganglia submucous and myenteric plexus of intest wall cerebrovascular and general organ nerves	7	Stimulates small intestinal secretion. Inhibits gastric acid production Relaxes gallbladder Stimulates pancreatic secretion Vasodilatory agent lowers blood pressure Disturbs glucose and calcium metabolism	Diarrhoea* flushing attacks* diabetes hypercalcemia inhibited gastric acid secretion* hypotonia*	4 5

* Symptoms present in our patient

has been present in excessive amounts in many patients (6) but not in all (26). The novel finding in our subject with a typical WDS was the demonstration of a new spectrum of peptides in the pancreatic tumour including neurotensin, enkephalin and calcitonin. VIP could not be demonstrated in the tumour cells while high levels of the peptide were found in plasma.

The symptoms in our patient can be accounted for by the known effects of VIP alone (Table II) although some symptoms could also be related to

the other peptides. The raised plasma glucose, low gastric acid secretion may have been due to high neurotensin level which has been shown to exert such effects on rats (2, 9). The discrepancy between the high VIP level in the blood and the inability to demonstrate VIP-producing cells in the tumour is probably due to technical difficulties in the immunohistochemical procedure. VIP could also have been released from sites outside the tumour by substance(s) produced in it. Calcitonin is probably an unlikely candidate for such a role.

ents with diarrhoea and high calcitonin levels in medullary thyroid carcinoma recently were found to have normal levels of VIP in the circulation (34). The effects of enkephalin and neurotensin on VIP in plasma are still unknown. In this context it was of special interest that Grimelius staining technique showed cells in the tumour that did not stain with any of the antibodies used. The persisting high PP values after surgery could be due either to some other tumour not observed or to hyperplasia of islet PP cells outside tumour tissue as described by Larsson (10). Patients with endocrine pancreatic tumours, especially those with diabetes mellitus, have been found to have raised PP levels which correlate with the level of blood glucose (19). Normalization of blood glucose in our subject and still persisting high values of PP make this aetiology less probable. The development of hypertension after surgery accords with previous findings in similar patients. This may be a prolonged rebound effect after normalization of the VIP levels when the tumour has been removed.

Our patient has been described with WDS and a pancreatic tumour containing immunoreactivity to phalloidin, neurotensin and calcitonin. In addition, elevated levels of VIP were also found but only in the plasma. This is the first time that neurotensin has been demonstrated in a pancreatic tumour. However, neither neurotensin nor enkephalin alone could explain the florid collection of symptoms described in this patient.

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Nonoperative Localization of Aldosterone-Producing Adenomas

An Analysis of the Efficiency of Different Diagnostic Procedures Made from 11 Cases and from a Review of the Literature

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CT. The efficiency of six different methods for topographical diagnosis of primary aldosteronism was analysed in retrospect from 11 surgically verified cases and by an analysis of 360 cases cited in the literature. Differentiation between hyperplasia and adenoma was most safely predicted by monitoring the diurnal rhythm of plasma aldosterone and its reaction to posture. Adrenal vein catheterization with aldosterone determinations was useful in establishing the presence of hyperplasia in approximately 70% of these cases. Correct localization of aldosterone producing adenomas was made in more than 90% by vein catheterization while venography and radio-cholesterol scintigraphy had lower diagnostic accuracy. It is therefore suggested that determination of plasma aldosterone both by vein catheterization and by peripheral monitoring should be used ahead of other diagnostic procedures for topographical examinations in primary aldosteronism.

Key words: aldosterone, ¹³¹I cholesterol scintigram, catheterization.

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Primary aldosteronism (PA) is a primary cause of increased blood pressure in less than 1% of patients with hypertension (20). A correct diagnosis of this syndrome is however of great importance since the appropriate therapy leads to improvement or permanent cure from the hypertensive and metabolic consequences of aldosterone hypersecretion. The diagnosis of PA is usually established with difficulties thanks to widely available sensitive specific methods for determination of aldosterone and renin. The major problem at present is to detect the morphological substrate for the disease: unilateral or bilateral adenoma or bilateral hyperplasia. Several diagnostic methods are being used for this purpose. Thus angiographic examina-

tions of adrenal arteries or veins have been used over more than decades (7, 8, 24). ¹³¹Iodine-cholesterol scintigram was introduced by Conn's group in 1971 (6) and was thereafter modified by simultaneous administration of dexamethasone (36) as well as by the introduction of new iodine cholesterol compounds (23, 33). Determination of aldosterone in plasma sampled from the adrenal veins was described by Bucht et al. in 1964 (4) and has since then been extensively used (7, 14, 16, 29, 35, 39, 42). Finally it has been suggested that the pattern of the diurnal rhythm of plasma aldosterone as well as the effect of posture on plasma aldosterone would be of value for the distinction between hyperplasia and tumour (1, 15, 19, 34).

The aim of the present study was to evaluate the efficiency of the above diagnostic procedures for the localization of aldosterone producing adrenal tumours and for the non operative distinction between tumours and hyperplasia.

PATIENTS AND METHODS

Patients

Unilateral adrenalectomy due to PA was performed in 11 subjects in 1975-78 at the Karolinska Hospital, Stockholm. All had a history of hypertension (1-19 years) and hypokalemia. The diagnosis was established by the demonstration of a low plasma renin activity (PRA) together with a high urinary excretion of aldosterone not suppressible by exogenous administration of mineralocorticoids (Table II). In ten of the subjects a typical adrenal tumour was removed at surgery followed by correction of hypokalemia and by improvement of or cure from hypertension. In one subject (no. 1) no tumour was found at operation. Adrenalectomy was performed on the left side and the microscopic examination revealed a diffuse

Abbreviations: PA = primary aldosteronism; CT = computer tomography; PRA = plasma renin activity.

Table I Relevant data on the patients

Case no	Age (y)	Sex	Duration of hypertension (y)	BP (mmHg)	Lowest serum K ⁺ (mmol/l)	Recumbent/upright PRA (pkat/l)	Urinary aldosterone excretion basal/during Florinef [®] (nmol/day)	PAD
1	67	♂	12	240/135	2.8	0.07/0.22	84/100	Hype
2	58	♀	9	205/110	2.9	0.04/0.04	100/90	Aden
3	55	♀	16	215/110	2.2	0.01/0.01	55/50	Aden
4	54	♀	18	210/110	2.8	0.02/0.04	55/60	Aden
5	52	♀	19	200/110	2.2	0.04/0.04	140/170	Aden
6	50	♀	15	240/140	1.8	0.04/0.04	205/120	Aden
7	48	♂	7	235/140	3.1	0.02/0.05	115/115	Aden
8	41	♀	11	220/115	2.4	0.01/0.01	55/60	Aden
9	39	♀	5	180/110	2.2	0.02/0.02	160/140	Aden
10	38	♂	1	185/125	2.9	0.04/0.10	145/120	Aden
11	22	♀	2	180/105	2.3	0.02/0.04	105/85	Aden

* Mineralocorticoid suppression test was performed over four days with fludrocortisone (Florinef[®]) in a dose of 0.4 mg. Aldosterone levels on the last day of the test are given.

hyperplasia of the zona glomerulosa. Hypokalemia and hypertension persisted in this case after the operation.

Diagnostic methods

Adrenal arteriography, aortography and celiac arteriography were performed in nine cases (24). Adrenal venography was performed in all 11 cases. Catheterization was done with a preformed radiopaque catheter. Only A/P projections were used.

In connection with venography but prior to the complete injection of contrast medium, blood samples were collected from caval and left adrenal veins for determination of aldosterone and cortisol. When possible, samples were also drawn from the right adrenal vein.

¹³¹I cholesterol scintigrams were carried out by a slight modification of the method described by Seabold et al (36). In order to block the isotope uptake in the thyroid gland we have given both levothyroxine 0.2 mg daily (Levaxin[®] Nyegaard, Oslo, Norway) and Lugol's solution for 14 days prior to isotope administration. Dexamethasone 2.0 mg daily (Dexacortin[®], Organon, Oss, Holland) was given for nine days except for cases 2, 3 and 11 who received steroids for three days only. The adrenal uptake of radioactivity was counted by a gammascintillator at three and seven days after the isotope injection.

Computer tomography (CT) was performed in five cases. A Delta scanner with an exposure time of 150 sec was used in four of them. In case 7 CT was done by a General Electric 7800 with an exposure time of 5.6 sec.

The diurnal rhythms of aldosterone and cortisol were assessed by venous blood sampling at 06, 12, 16, 22 and 02 hours. Plasma aldosterone was determined according to Poulsen et al (32) and cortisol by a fluorometric method (30).

RESULTS

Adrenal arteriography (Table II)

Adrenal arteriography was performed in eight subjects with unilateral adenoma. The adrenals were

visualized on both sides in four patients and side in two. In two cases the adrenals were not visualized. Thus the procedure was technically successful in 10 of 16 examined adrenal glands. In the hyperplasia patient, arteriography revealed normal adrenals. Out of six tumours in a group of 16 patients, four were adequately visualized at arteriography and two were not seen, whereas two were too small to be detected by this method.

Adrenal venography (Table II)

The left adrenal was visualized in all ten patients with unilateral tumour while the right adrenal was not visualized in five of them. Four out of six tumours in the left adrenal gland and one out of four in the right were visualized at venography. Those not visualized were small ones (cases 7 and 11). In patient 9 a large tumour was found on the left side. In this patient, however, venography indicated a tumour on the left side. Full remission of hypertension and potassium depletion following removal of the right adrenal gland proved the left-sided lesion was not an aldosterone-producing tumour. Thus in this patient the venography examination gave false positive information. In the patient with hyperplasia, venography disclosed a normal adrenal gland at the left side. Catheterization of the right adrenal vein failed.

¹³¹I cholesterol scintigram (Table III)

Adrenal scintigrams during dexamethasone suppression were carried out in all patients. The correct localization was achieved in seven patients.

II Localization of adrenal tumours by adrenal angiography

not visualized NP = not performed

				Findings at exploration	
Venography		Arteriography		Approximate tumour weight (g)	Localization of tumour
sin	dx	sin	dx		
Tumour	Normal	Tumour	NV	2	sin
Normal	NP	Normal	Tumour	8	dx
Tumour	NP	Tumour	NV	3	sin
Normal	NP	NV	NV	4	dx
Normal	NP	Normal	Tumour	10	dx
Normal	Normal	NP	NP	1	sin
Tumour	NP	NP	NP	1	sin
Tumour	Tumour	NV	NV	6	dx
Tumour	Normal	Normal	Normal	<1	sin
Normal	Normal	Normal	Normal	<1	sin

Prosthetic results

	No. of angiographies performed			
	10	5	8	8
Correct	7	5	4	4
False positive	1			
False negative	2		2	
Inclusive			2	4

1 adenoma. In two cases (nos 8 and 19) no ope uptake was registered and in case 7 bilateral ope uptake of similar magnitude was seen. In patient with hyperplasia the scintigram indicated correctly bilateral autonomous aldosterone production (11).

Computer tomography

Of four adenoma patients examined by CT the tumour was detected in only one (no 6) who had largest tumour (Table II). Though the tumour of no 3 was quite large it was not identified by CT. In the subject with hyperplasia the adrenals appeared normal on CT.

Adrenal vein catheterization (Table IV)

This investigation was performed in eight patients with adenoma and in the patient with hyperplasia. In case 7 the procedure was repeated after one month. The left adrenal vein was successfully explored in all investigations while adequate blood samples were obtained from the right side on only two occasions.

Cortisol in samples from vena cava varied be-

tween 120 and 880 nmol/l and in those from adrenal veins from 220 to 9270 nmol/l. In general the plasma cortisol levels were several times higher in adrenal than in systemic blood. There was not significant difference between cortisol levels in samples

Table III Isotope uptake in adrenals during ^{131}I cholesterol scintigram

— = No uptake + = slight uptake ++ = high uptake

Pat no	Isotope uptake in adrenals		PAD
	sin	dx	
1	+	+	Hyperplasia
2*	++	+	Adenoma sin
3	—	++	Adenoma dx
4	+	—	Adenoma sin
5	—	++	Adenoma dx
6	—	++	Adenoma sin
7	+	+	Adenoma sin
8	—	—	Adenoma sin
9	—	++	Adenoma dx
10	—	—	Adenoma sin
11	++	+	Adenoma sin

* Dexamethasone administration for three days only

Table IV Plasma levels of aldosterone (pmol/l) and cortisol (nmol/l) measured at certain places in venous system by gentle catheterization

Subj no	Left adrenal vein			Right adrenal vein			Vena cava			PAD
	Aldosterone	Cortisol	Ratio*	Aldosterone	Cortisol	Ratio*	Aldosterone	Cortisol	Ratio*	
1	4 770	1 780	2.7			—	2 170	410	5.1	Hyperplasia
2	44 000	750	59.3	7 000	9 270	0.8			—	Adenoma
3	600	740	0.8			—	1 300	360	3.6	Adenoma
6	1 700	750	2.3			—	8 500	270	31.5	Adenoma
7 ^b	7 600	340	22.4			—	2 000	510	3.8	Adenoma
7 ^b	111 000	3 000	36.3			—	1 800	490	3.7	Adenoma
8	24 000	5 980	4.1			—	2 000	880	2.3	Adenoma
9	4 400	1 850	2.4	83 000	2 370	37.2			—	Adenoma
10	8 700	1 370	6.3			—	5 300	790	6.7	Adenoma
11	18 000	610	29.3	500	220	2.2	600	120	5.0	Adenoma

* Calculated as $\frac{p \text{ aldosterone (pmol/l)}}{p \text{ cortisol (nmol/l)}}$

^b Catheterized on two occasions

from the adrenal veins of five normal glands (2570 ± 1700 nmol/l mean \pm S.E.M.) and from six tumours (2070 ± 750 nmol/l) indicating that the cortisol secretion measured locally by crude sampling was not changed by the presence of an aldosterone producing tumour.

In the three adenoma cases in whom a successful bilateral catheterization was performed (nos 2, 9 and 11) 6–20 times higher aldosterone levels were found in plasma sampled from the adrenals with tumours. In the other five adenoma patients a comparison was made between the aldosterone levels in the left adrenal vein and the caval vein. In cases 3 and 11 the levels in the caval vein were much higher than in the left adrenal vein which strongly suggested hypersecretion of aldosterone from the right adrenal. In cases 7, 8 and 10 aldosterone levels were higher in the left adrenal vein than in the caval vein. In all these patients the adenoma was found in the left adrenal gland. In patient 7 the aldosterone level in the left adrenal vein was almost ten times higher during the second than the first catheterization whereas the ratio between aldosterone and cortisol was almost the same. This suggests that a quotient between aldosterone and cortisol may offer a better estimation of the true secretion of aldosterone.

In the subject with bilateral hyperplasia only the left adrenal vein was catheterized. The aldosterone/cortisol ratio for this subject was higher in caval vein than in the left adrenal vein suggesting high secretion of aldosterone from the right adrenal.

Thus in this case the diagnosis of hyperplasia could not be established by venous sampling alone.

Diurnal rhythm and effect of posture on peripheral plasma aldosterone and cortisol levels (Table V)

In all eight subjects examined plasma aldosterone at 6 a.m. was clearly above the normal range (272 pmol/l) while plasma cortisol was within normal range (276–828 nmol/l). The seven adenoma patients and the patient with hyperplasia had a fully normal diurnal rhythm of cortisol and aldosterone with nadir concentrations in the late afternoon and a rise in the concentrations during the morning hours. The effect of posture on aldosterone secretion was analysed by comparing peripheral concentrations in recumbent position at 6 a.m. with those at noon, i.e. after at least 10 hours in the upright position. A fall in plasma aldosterone was noticed in all cases with PA due to adenoma. In the patient with bilateral hyperplasia (no 1) a rise in plasma aldosterone was seen upon posture.

Efficiency of diagnostic procedures analysed in review of 360 operated cases of PA reported in the literature (Table VI)

In 38 reports during recent years 452 patients with PA have been presented. A unilateral adenoma was found in 309 of the 360 operated cases. In 17

Table V Diurnal variation in plasma cortisol (nmol/l) and aldosterone (pmol/l) in patients with PA due to lateral adenoma or hyperplasia

subjects were in recumbent position between 22 p.m. and 08 a.m.

	Measured at				
	06 a.m.	12 00	16 p.m.	22 p.m.	02 a.m.
<i>hyperplasia</i>					
Cortisol	490	340	190	60	130
Aldosterone	612	837	879	403	153
<i>lateral adenoma</i>					
Cortisol	410	420	500	190	260
Aldosterone	1 570	620	510	120	1 070
Cortisol	427	176	99	187	127
Aldosterone	693	471	388	388	444
Cortisol	529	309	151	85	309
Aldosterone	4 051	943	888	1 637	3 108
Cortisol	490	380	180	170	170
Aldosterone	1 084	456	346	363	640
Cortisol	436	356	346	179	140
Aldosterone	1 359	471	610	249	693
Cortisol	570	470	190	220	220
Aldosterone	1 986	1 786	858	1 648	1 889
Cortisol	402	187	201	121	55
Aldosterone	1 387	610	471	555	388

ve 309 the affected side was denoted in the report concerned. Thus 61% of the tumours were localized to the left side. A total of 101 cases were considered to have bilateral hyperplasia. However only 48 subjects this diagnosis was verified by microscopic examination. As in the adenomas only surgically verified cases were included in the calculations. In the hyperplasia cases however it was always possible to exclude microscopically unverified cases since the reports do not regularly give this information.

Although no group of workers has used all the diagnostic methods applied in our study some groups had compared the diagnostic value of two or three methods. Thus in patients with adenoma the correct tumour localization was usually best predicted by estimating the plasma aldosterone concentration in the adrenal vein by adrenal vein catheterization procedures in spite of the fact that the adrenal veins were successfully catheterized in less than 50% of the cases (14, 21, 29, 35, 36, 39).

Thus the diagnostic efficiency of this method as a tumour localizer was excellent predicting the correct side in 93% of the cases. In cases with bilateral hyperplasia the accuracy of this method

was somewhat lower a correct diagnosis being predicted in about 74% of the cases.

Adrenal venography has been extensively used and at least some groups of researchers have obtained very good results. Thus Conn et al (8) have reported a correct tumour localization in 32 out of 38 adenoma cases. However this method has given less satisfactory results according to other investigators. This is mainly due to the difficulties to catheterize the right adrenal vein. As to the summarized experience with venography a correct diagnosis was predicted in about 62% of the cases. It is noteworthy that here too one false positive result has been reported (21).

Adrenal radio cholesterol scintigraphy was originally introduced in 1971 by Conn's group (6). In 1976 they reported on their results with this method in 16 patients with unilateral adenoma and five with bilateral hyperplasia. Based on mainly the same subjects Seabold et al (36) demonstrated a unilateral isotope accumulation in about 80% of the adenomas. In contrast to these reports Fukuchi and Nakajima (14), Helber et al (17) and Yune et al (42) have reported on a much lower diagnostic efficiency in adenoma cases. As to patients with

Table VI Survey of 360 operated PA cases collected from the literature

Authors	Year	Adenoma correctly localized by				Hyperplasia correctly localized by			
		Veno graphy	Cathe- teriza- tion	Radio choles- terol scinti- graphy	Decrease in plasma aldoste- rone in upright position ^a	Veno graphy	Cathe- teriza- tion ^a	Radio choles- terol scinti- graphy	Dec- rease in p- aldoste- rone in up- right posi- tion ^a
Balikian et al (1)	1968	-	-	-	0/3	-	-	-	-
Britton et al (3)	1976	1/2	2/2	2/2	-	-	-	-	-
Clarke et al (5)	1979	2/7	7/7	5/6	2/4	0/1	1/1	0/1	0/1
Conn et al (7, 8)	1972-76	32/48	-	2	-	-	-	-	-
Dige Petersen et al (9)	1975	-	-	2/3	-	-	-	-	-
Espinosa et al (11)	1976	5/7	6/10	-	-	-	-	-	-
Fromantin et al (13)	1977	0/3	3/3	1/1	-	-	-	-	-
Fukuchi et al (14)	1976	7/22	18/23	19/27	-	-	-	-	-
Ganguly et al (16)	1973	-	7/7	-	9/11	-	2/4	-	0/1
Helber et al (17)	1975	-	-	3/5	-	-	-	-	-
Hogan et al (18)	1976	-	-	8/8	-	-	-	-	-
Horký et al (19)	1976	-	4/4	-	4/4	-	2/2	-	0/1
Horton & Finck (21)	1972	8/12	12/12	-	-	2/2	2/2	-	-
Mantero et al (26)	1976	-	-	-	5/5	-	-	-	0/1
Melby et al (28)	1967	3/7	7/7	-	-	-	-	-	-
Mitty et al (29)	1973	6/10	8/8	-	-	-	-	-	-
Ohbuchi & Murakami (31)	1976	-	-	-	7/9	-	-	-	-
Sarkar et al (33)	1977	4/4	4/4	4/4 ^c	-	1/4	4/5	3/5 ^{c,f}	-
Schambelan et al (34)	1976	-	-	-	6/6	-	-	-	0/1
Scoggins et al (35)	1972	3/10	10/10	-	-	-	-	-	-
Seabold et al (36)	1976	11/14	9/9	10/13 15/16 ^d	-	1/4	3/3	1/5 5/6 ^d	-
Sundsfjord et al (37)	1974	-	-	2/2	-	-	-	-	-
Vetter et al (40)	1974-76	-	9/9	8/9	2/5	-	0/2	0/2	0/1
Wiggins et al (41)	1976	-	4/4	-	-	-	-	-	-
Yunc et al (42)	1976	8/10	8/8	3/8 ^e	-	-	-	-	-
Correct information of total no of investigations (%)		62	93	79 75 ^a	74	-	74	-	-

^a Blood taken from adrenal vein(s) for analysis of aldosterone and cortisol concentrations^b Peripheral blood samples taken in the morning (recumbent) and at noon (upright position)^c Patients included in the report by Seabold et al^d Two of reported cases verified by surgery^e One of reported cases verified by surgery^f ¹²⁵I-β-iodomethyl 19 nor-cholesterol scintigraphy performed with dexamethasone suppression^g Diagnosis not confirmed by surgery^h Performed under dexamethasone suppression

hyperplasia Conn et al (7) and Seabold et al (36) have reported on a high diagnostic efficiency by this method. This has however not been confirmed by other investigators (17-42). Thus based on the present experience as calculated from the literature ¹²⁵I-cholesterol scintigraphy gives false positive information in more than 20% of the investigations.

Several groups have reported on a parallelism between the diurnal rhythms of cortisol and aldosterone in patients with PA due to adenoma while

such a parallelism was not seen in hyperplasia (15, 22, 26, 31, 39). Moreover Ganguly et al reported that plasma aldosterone concentration reduced in the majority of adenoma cases a few hours in upright position compared with hormone levels in recumbency. In contrast changed or even increasing plasma aldosterone concentrations were registered in hyperplasia cases. These observations have later on been confirmed by several investigators (19, 26, 31, 34). According to these reports no single subject

hyperplasia (12 verified by surgery) exhibited a gradual fall in plasma aldosterone concentration as this was seen in 77% of adenoma cases. In patients with PA due to adenocarcinoma Seabold (36) have reported on the correct localization of tumour in one case with venography and adrenal vein catheterization while radio cholesterologram did not visualize the metastatic masses. We have observed one case of PA due to a metastatic adrenal carcinoma in our clinic. Interestingly in this case the metastases did not accumulate enough to make their localization possible. In the very few carcinoma cases studied so far plasma aldosterone levels did not show diurnal rhythm or postural decrease (34-39).

DISCUSSION

In order to choose the best therapy for patients with a correct topographical diagnosis and differentiation between adenoma and hyperplasia is necessary.

The current opinion considers surgery unwarranted in patients with bilateral hyperplasia who should receive medical treatment with spironolactone or other drugs. On the other hand in patients with adenoma the surgical removal of an aldosterone producing tumour leads to normalization of blood pressure in about 70% of the cases (25).

The ideal method to solve the problems of topographical diagnosis in PA should be relatively inexpensive and associated with as little discomfort or adverse reactions for the patients as possible. Furthermore it should answer the two important questions: 1) Bilateral or unilateral adrenal disease? 2) In cases with unilateral disease to lateralize the tumour. Since numerous diagnostic procedures have been used in most reports on this subject one could envisage that this ideal method is lacking.

Of the radiologic methods adrenal venography has been most commonly used and some groups have reported on correct lateralization in 70-80% in cases with unilateral adenoma. In our opinion however the problems associated with catheterization of the right adrenal vein makes this method useful in only a limited number of cases. Furthermore adrenal venography is potentially dangerous as the rapid injection of contrast medium in 10% of patients leads to an extravascular contrast leakage (27). This complication was also found in two of our cases though not associated

with a serious outcome. Occasional infarction of an adrenal tumour may be fortuitously provoked in this way (12) and infarction of both adrenal glands producing adrenal insufficiency has been reported in at least two patients (10-38).

Thus morbidity after venography is not infrequent and even fatal outcome has been reported in one case (3). Another problem with radiological methods is that the demonstration of a tumour in a PA patient does not prove that the lesion is an aldosterone producing adenoma. Thus in accordance with Horton and Finck (21) and in one of our cases a tumour was seen in one adrenal without evidence of an enhanced steroid hormone production from this gland.

Adrenal arteriography has not been extensively used in patients with PA. From our experience the diagnostic efficiency of this method is not encouraging probably because most tumours causing PA are too small to allow identification. Thus inconclusive results are to be expected in 30-50% of the cases examined with arteriography. It is therefore our impression that adrenal arteriography should be used only if other diagnostic procedures have failed.

The radio cholesterol methods for visualization of the adrenals have received much enthusiasm. Our experience as well as that of some other groups indicates however that a correct lateralization is obtained in approximately more than 70-80% of the patients. This method is not free from complications either. In one of our cases the relatively long dexamethasone period had to be discontinued due to a florid psychosis a few days after the start of steroid administration.

There is no doubt that adrenal vein catheterization is superior to other methods in cases with unilateral tumours. Thus when both adrenal veins are successfully catheterized the correct topographic diagnosis can be safely predicted. However for technical reasons the right adrenal vein is difficult to catheterize. In most cases the interpretation has therefore to rely on blood samples taken from the left adrenal vein and from the inferior vena cava. Two major problems are associated with this interpretation. Firstly in our study as well as in other studies (16-28-41) there was an obvious overlap in the absolute concentrations of aldosterone in the adrenal vein from tumours hyperplastic and even normal glands. Secondly secretory bursts of aldosterone and cortisol from a diseased or normal

gland can occur during catheterization. Since no evidence is at hand demonstrating altered cortisol secretion in hyperplastic or tumorous glands, an improvement of the diagnostic precision can be achieved by calculating the ratio between plasma concentrations of aldosterone and cortisol. This is also demonstrated by us as well as by others (35). The ratio adrenal vein aldosterone to cortisol may therefore be of critical value when the catheterization procedure is successful on one side only.

The adrenal vein catheterization procedure allows no complete differentiation between PA due to adenoma or hyperplasia. It should also be noticed here that bilateral tumours have been found in about 10% of PA and that the differentiation from hyperplasia by vein catheterization in such a case will be very difficult. In many cases a discrimination between patients with adenoma and hyperplasia can be obtained by studying the postural effect on peripheral plasma levels of aldosterone and PRA. Although hyperplasia was found in only one of our patients, our findings were in good agreement with those of previous investigators. Thus, in this patient an increase in the plasma aldosterone concentration was noticed after posture while the hormone levels decreased in most of our adenoma cases. Also the PRA in this patient was slightly increased at standing up while no such effect was seen in the adenoma cases.

Therefore a correct preoperative topographic diagnosis can be made in most patients with PA with the adrenal vein catheterization procedure together with an analysis of plasma aldosterone in recumbent and upright position. In our opinion other diagnostic procedures such as adrenal venography, arteriography and radio cholesterol scintigraphy are of a more limited value. Furthermore the use of these methods is associated with adverse reactions. Therefore they should be used as diagnostic procedures only in patients in whom catheterization and peripheral hormone monitoring have failed. Computer tomography may be valuable in cases with large tumour masses i.e. malignant tumours. Most benign aldosterone producing tumours seem too small to allow detection by this new method.

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Amyloid Deposits in Bone Marrow Aspirates in Primary Amyloidosis

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STRACT Amyloid deposits aspirated from the bone marrow have a characteristic appearance in Papanicolaou-Giemsa stained smears, and a characteristic fibrillar pattern when viewed in an electron microscope.

Keywords: amyloidosis, bone marrow findings.
Acta Med Scand 208 111-113 1980

There seems to be some disagreement in the literature as to the frequency of finding amyloid in bone marrow aspirates from patients with primary amyloidosis. Hallen and Rudin (3) studied 18 patients with proven primary amyloidosis and did not find amyloid at histological examination of the bone marrow in any of them. Neither are amyloid deposits in the bone marrow mentioned in some of the leading textbooks of haematology nor pictured in any of the foremost haematological atlases. On the other hand, Conn and Sundberg (2) described amyloid in microphotographs of amyloid in bone marrow aspirates in 4 out of 5 patients with amyloidosis, and Kyle et al. (4) found amyloid in bone marrow smears in 16 out of 66 patients. We have recently seen a large number of amyloid deposits in bone marrow aspirates from two patients with primary amyloidosis.

CASE REPORTS

Case 1
A male born in 1923 was admitted to hospital in February 1975 with a more than 5 years' history of back pain. His height had reduced from 172 cm at age 20 to 161 cm in 1975, and roentgenograms had shown severe osteoporosis and several compression fractures of the vertebrae.

Laboratory findings on admission: Urinalysis was normal. ESR (Westergren) 49 mm/hour. Hb 13.9 g/100 ml. RBC 4.6 mill/ μ l. WBC 5100/ μ l. Platelets 175 000/ μ l. Serum concentrations of calcium 2.4 mmol/l (normal 2.2-2.6), phosphorus 1.2 mmol/l (normal 0.9-1.4), creatinine 89 μ mol/l (normal 71-124).

Total serum proteins were 71 g/l and serum electrophoresis was normal. Bone marrow aspirate, liver biopsy and aspirated subcutaneous fat all showed amyloid deposits. Test for serum amyloid A related protein was negative. The bone marrow aspirate showed on the average 4% mature looking plasma cells. A diagnosis of primary amyloidosis was made.

Case 2

A female born in 1918 was admitted to hospital in November 1975 because of lassitude and proteinuria.

Laboratory findings: Urinalysis showed moderate proteinuria 2.7 g/l. ESR (Westergren) 133 mm/hour. Hb 11.2 g/100 ml. RBC 4.0 mill/ μ l. WBC 6400. Platelets 282 000/ μ l. Serum concentrations of calcium 2.2 mmol/l, phosphorus 1.5 mmol/l, creatinine 146 μ mol/l.

Total serum proteins were 59 g/l. Serum electrophoresis showed albumin of 20 g/l. There was a monoclonal component of about 10 g/l in the beta-gamma region. Immune electrophoresis showed the monoclonal component to be IgA lambda.

Agarose and immunoelectrophoresis of the urine showed moderate non-selective proteinuria which included polyclonal IgG and small amounts of IgA lambda. Bence Jones proteinuria was not found. Bone marrow aspirate and renal biopsy showed amyloid deposits. The bone marrow aspirate showed 10% somewhat immature plasma cells and renal biopsy showed some tubular casts of lamellar appearance. A diagnosis of myelomatosis with amyloidosis was made.

MATERIALS AND METHODS

Light microscopy: Films from bone marrow aspirate were fixed in absolute methanol and then stained by May-Grunwald-Giemsa at pH 6.8.

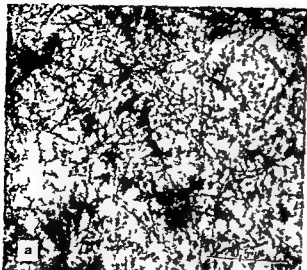


Fig 2 Electron microscopical section from bone marrow of case 1 (a) and case 2 (b) showing the characteristic meshwork of amyloid fibrils

Electron microscopy The bone marrow aspirate was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2-7.4 for 2 hours and postfixed in 1% osmium tetroxide in Tyrode solution pH 7.4 for 1 hour. Dehydration was carried out in graded ethanols and the specimen was embedded in Epon 812. The sections were cut with an LKB Ultramicrotome, stained with lead citrate and uranyl acetate and examined in a Siemens Elmiskop I electron microscope.

RESULTS

Light microscopy The bone marrow smears from case 1 showed a large number and from case 2 a moderate number of pink to purple waxy to transparent irregularly shaped masses. These masses which often surrounded and incorporated a large number of bone marrow cells might have with a hasty glance been mistaken for ordinary marrow particles. In some places the masses did not incorporate any bone marrow cells and then had a quite characteristic appearance (Fig. 1b, colour plate). The marrow of case 1 contained 4% mature plasma cells and of case 2 10% somewhat immature plasma cells.

Electron microscopy At a magnification of about 60 000 we could see a meshwork of fibrils with a width of approximately 7.5-10 nm (Fig. 2). This appearance is typical for amyloid deposits.

DISCUSSION

Our patient 1 had severe osteoporosis and always resembled a patient described by Axelsson (1). Our patient showed large amorphous amyloid deposits in all bone marrow aspirates, the sternum as well as from the iliac crest. There were about 4% plasma cells in the bone marrow without signs of immaturity. Test for serum amyloid A related protein was negative. A diagnosis of primary amyloidosis was made. Patient 2 had nephrotic syndrome, monoclonal IgA lambda in serum and 10% somewhat immature plasma cells in the bone marrow. A diagnosis of multiple myeloma with amyloidosis was made.

The appearance of amyloid deposits in W stained bone marrow smears has been described by Conn and Sundberg (2). A structureless homogeneous purple to pink staining material occurring in masses or clouds among marrow cells. The masses of amyloid are discrete or occur in association with clusters of cells, fat or fragments of vessels. Isolated masses of amyloid give a distinctive cumulus cloud-like appearance, appearing light and transparent near the edges and more dense and billowy near the center. This description fits well also our cases.

In our patients the electron microscopy, showing a meshwork of typical amyloid fibrils, proved that the structureless pink to purple

the bone marrow really were amyloid deposits. We think that it is important to become aware of the characteristic appearance of amyloid deposits when found in smears from bone marrow aspirates. In some cases it may (perhaps unexpectedly) come an important lead towards the correct diagnosis and may explain an otherwise puzzling histological finding.

ACKNOWLEDGEMENT

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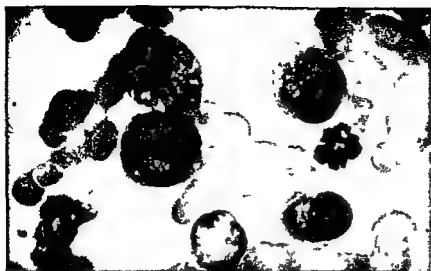


Fig 1a Plasma cells showing some irregularities in the cytoplasm which perhaps correspond to concentric lamellar bodies (May-Grunwald-Giemsa)



Fig 1b Smear of bone marrow aspirate showing amyloid desposits (May-Grunwald-Giemsa)

Abnormal Pattern of the Rough Endoplasmic Reticulum of Plasma Cells in Multiple Myeloma with Multiple Concentric Lamellar Bodies and "Single Sac Loops"

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ABSTRACT A patient with IgG myeloma showed a very abnormal pattern of the rough endoplasmic reticulum (RER) with multiple concentric lamellar bodies and "single sac loops". This abnormal pattern may be due to either a complete rearrangement of the architecture within the cell and/or a pathology in the wall of the sacs of RER.

Keywords: myeloma, abnormal RER pattern.
Acta Med Scand 208: 115, 1980.

It is (1) quoted a number of different types of morphology of the rough endoplasmic reticulum (RER) reported in multiple myeloma. In most instances a fairly large number of pathological plasma cells are reported in a particular patient. Most patients with multiple myeloma have a well developed RER, but occasionally it may be represented by a few lamellae and has a more lymphoid appearance.

Alonzo et al. (3) and later Tanaka and Good (4) described various patterns of RER in multiple myeloma: lamellar, tubular, concentrically arranged around the nucleus, lamellar tubular concentrically arranged around nucleus or vesicular instead of lamellar. In some cases the tubules or vesicles would be moderately or markedly distended. The markedly distended sacs of the RER may contain soluble proteins which appear as flaming in plasma cells and solid proteins appearing as Russell bodies when stained with May-Grunwald-Giemsa (1). We have recently observed a pattern of multiple concentric lamellar bodies and single sac loops in the RER of the plasma cells in a patient with multi-

CASE REPORT

The patient was a female born in 1918. In Dec. 1974 she had a transitory febrile period lasting for a few days. Her family doctor found an increased ESR which remained elevated around 120 mm/hour. Serum electrophoresis in Feb. 1975 showed a monoclonal gammopathy.

The patient was admitted to the Section of Haematology, Medical Department A, Rikshospitalet on April 22, 1975. On admission she was moderately pale, otherwise normal findings. Laboratory findings: ESR 135 mm/hour, Hb 9.7 g/100 ml, leukocytes $2.6 \times 10^9/l$, platelets $238 \times 10^9/l$, blood. There were 20% plasma cells in the bone marrow. Total serum proteins were 110 g/l. A large monoclonal (IgG kappa) component was found in the gamma region and gamma globulins were 49 g/l. No osteolytic lesions were observed. Serum creatinine was normal.

The patient was treated with melphalan and responded. The total proteins decreased gradually to 78 g/l in Oct. 1976 with a gammaglobulin concentration of 20 g/l. She relapsed again and in Aug. 1977 the total proteins were 124 g/l, gammaglobulin 58 g/l. The bone marrow showed 50% somewhat immature plasma cells. She gradually developed osteoporosis with compression fractures of the spine and anaemia palliated with transfusions. She did not respond to treatment with cyclophosphamide, vincristine and prednisone given between Aug. 1977 and April 1978 or to vincristine, cyclophosphamide, BCNU, melphalan and prednisone administered from April until Sept. 1978 when she died.

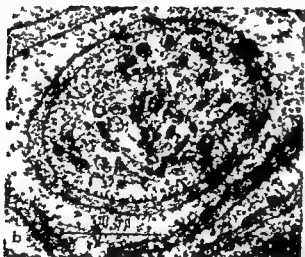
MATERIALS AND METHODS

Light microscopy. Films from bone marrow aspirate were fixed in absolute methanol and stained by May-Grunwald-Giemsa at pH 8.

Electron microscopy. The bone marrow aspirate was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2-7.4 for 2 hours and postfixed in 1% osmium tetroxide in Tyrode solution pH 7.4 for 1 hour. Dehydration was carried out in graded ethanol and the specimen was embedded in Epon 812. The sections were cut with an



Fig. 2 (a) Plasma cell nucleus with a prominent nucleolus and several concentric lamellar bodies (single sac loops) in the cytoplasm. The smooth endoplasmic reticulum of the apparatus (b) is showing the structure resembling the smooth endoplasmic reticulum of the Golgi apparatus.



but perhaps not more than usually seen myeloma (Fig. 1a colour plate)

Electron microscopy. An aspirate from bone marrow was taken on Jan 31 1978. The cells appeared immature with prominent nucleoli (Figs 2a and 3a). The RER was well developed, about 25% of the plasma cells. There was a normal pattern of the RER with multiple lamellar bodies as well as bulging of the flattened sac appearing as single sac loops (Fig. 3). In the centre of some of the lamellar bodies or single sac loops there were 3–15 vesicles of rough endoplasmic reticulum. In other centres there were structures resembling the smooth endoplasmic reticulum of the apparatus (Fig. 2). The ordinary Golgi apparatus in these cells was somewhat scanty. No Golgi apparatus could be seen in some of the centres (Fig. 3a).

LKB Ultramicrotome stained with lead citrate and uranyl acetate and examined in a Siemens Elmiskop I electron microscope

RESULTS

Light microscopy. On Jan 31 1978 the sternal marrow aspirate was of average cellularity. There were about 50% plasma cells with nucleoli and appearing moderately immature. The cytoplasm was basophilic. In some of the plasma cells the cytoplasm contained a few vacuoles and some irregularities.

DISCUSSION

Concentric lamellar bodies derived from the plasma reticulum have previously been seen in some normal cells and in a variety of pathologically altered cells (2), probably most often in liver of viral hepatitis hepatomas and after administration of various chemicals to animals. Concentric



3 (a) Plasma cell with two nucleoli and several saccular loops and concentric lamellar bodies at least of them with 3-15 vesicles of RER in the centres (b) showing a centre with some 15 vesicles of RER

lar bodies are occasionally found in plasma cells (3, 4). A portion of cytoplasm containing some organelles, particularly lipid droplets and mitochondria (?) is often sequestered at the centre of such bodies.

The RER of our patient contains multiple concentric lamellar bodies but also in many places a single flattened sac sectioned in a way resembling a loop, sometimes joined to another similar loop. These loops will correspond to a localized bulging of the still flattened sac (a single sac loop); this bulging being quite different from the distention of the sacs seen in flaming plasma cells or from Russell bodies.

In some of the centres of the concentric lamellar bodies of single sac loops, there are either a number of vesicles of RER (Fig. 3b) or structure resembling the smooth endoplasmic reticulum of the Golgi apparatus (Fig. 3b). This, in addition to a somewhat scanty normal Golgi apparatus, might suggest a complete rearrangement of the architecture in these cells. In other centres there are no central structures and the bulging of the sac might then be due to pathology in the wall of the sac or in its formation.

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Multifocal Idiopathic Fibrosclerosis

Two Cases with Simultaneous Occurrence of Retroperitoneal Fibrosis and Riedel's Thyroiditis

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TRACT The concomitant occurrence of retroperitoneal fibrosis (RF) and Riedel's thyroiditis is reported in two patients, one of whom presented with retroperitoneal fibrosis. The etiology is discussed, and it is concluded that immunological mechanisms with arteritis might be of importance. The associations between Riedel's thyroiditis, RF, mediastinal fibrosis, sclerosing cholangitis, retroorbital fibrosis and fibrosis in other organ systems are thought to be different manifestations of a so-called multifocal idiopathic fibrosclerosis. The beneficial effect of glucocorticoids in the early stages of the disease is mentioned.

Keywords: Riedel's thyroiditis, retroperitoneal fibrosis, multifocal idiopathic fibrosclerosis.

Med Scand 208 119 1980

Retroperitoneal fibrosis (RF) and Riedel's thyroiditis are both rare diseases with aggressive features, accompanied by infiltrates of inflammatory cells in the retroperitoneal space and the thyroid gland, respectively. RF as a nosological entity was first described in 1948 by Ormond (19). Until now, only 500 cases have been published (11). In Scandinavia, the first case was published in 1956 (18). In the original paper, Riedel described the invasive thyroiditis as "eisenhartes Struma" (22). The etiology of the disease was emphasized by Woolner & Jaffe (26), who found only 20 cases in 42,000 thyroidectomies.

In the present paper, we describe the association between RF and Riedel's thyroiditis, which has not previously been reported from Scandinavia.

CASE REPORTS

In March 1962, a 44-year-old previously healthy female was admitted to the Surgical Department because of pain and enlargement of the left thyroid gland with tracheal

compression symptoms for about 2 months. She had had no previous medication. The patient appeared euthyroid with an about hen egg-sized, very hard enlargement of the left lobe of the thyroid gland. The right thyroid gland was normal. The basal metabolic rate was normal. S-creatinine was 0.9 mg/100 ml (under 1.5 mg/100 ml). Carcinoma was suspected and a thyroidectomy was planned but had to be abandoned as the left lobe was severely infiltrated in the adjacent structures. A biopsy was performed instead. The postoperative course was uneventful and radiation therapy was given. During the following year, no further compression of trachea occurred and no increase was observed in the thyroid gland. S-creatinine was normal.

In Nov. 1963, the patient was admitted to the Medical Department because of anemia. Hb was 62 g/l. S-creatinine was 4.0 mg/100 ml. The diagnosis was chronic pyelonephritis.

Owing to progressive nephropathy, the patient was readmitted to the Medical Department in Sept. 1965. Her S-creatinine at that time was 11.4 mg/100 ml. A retrograde pyelography revealed hydronephrosis on both sides with obstruction and medial displacement of both ureters at the fourth lumbar level. At this time, the patient felt well and as the S-creatinine decreased to 8.5 mg/100 ml during 14 months in the hospital, no further investigation was carried out.

In Sept. 1967, the patient was referred to the Medical Department on account of lassitude, giddiness and polyuria. A right-sided pyelography revealed unchanged hydronephrosis and S-creatinine was 8.5 mg/100 ml. No proteinuria was found. RF was suspected and radiation therapy was given. Three months later, the patient's S-creatinine was 9.2 mg/100 ml. She was transferred to Aarhus Kommunehospital, where a laparotomy revealed that both ureters were encased in a hard fibrous mass from the level of the promontory and 6 cm distally. Ureterolysis was performed on both sides, but S-creatinine remained unchanged in spite of normal diuresis. The patient deteriorated slowly and died about 9 months later.

Further laboratory studies: From the first admission onwards, the patient had elevated ESR, ranging from 44 mm/h to 105 mm/h. The thyroid parameters were normal during the whole period. No elevation of thyroid globulin antibodies was found and no abnormality in serum protein electrophoresis was detected.

Pathology: Microscopic examination of the thyroid biopsy showed severe atrophy of the thyroid follicles with interstitial fibrosis and infiltration with lymphocytes, plasma cells and scattered eosinophils. No signs of malignancy were found.

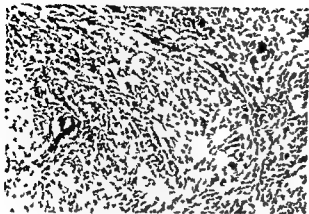


Fig 1 Case 1 The thyroid gland with severe fibrosis without remnants of follicles (H & E)

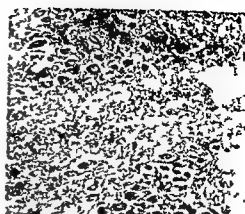


Fig 2 Case 1 Perithyroid tissue with severe splitting of the striated muscle fibres. There is lymphocyte infiltration (H & E)

nancy were found. Some small arteries showed intimal thickening and infiltration of the wall with lymphocytes. The conclusion was Riedel's thyroiditis (Figs 1-3).

Post mortem examination revealed a very hard fibrous mass in the retroperitoneal space extending from the promontory to the pelvis and obstructing both ureters. Hydronephrosis was found on both sides. The left thyroid gland had shrunk to a hard fibrous mass. The right thyroid gland was normal. Macroscopic examination of the thyroid glands revealed severe interstitial fibrosis of the left gland compatible with Riedel's thyroiditis. The retroperitoneal tissue was not examined microscopically. There was no sign of fibrosis in the other organ systems.

Case 2

A 62 year old previously healthy female was admitted to the Surgical Department in Dec. 1976 because of painless enlargement of the left thyroid gland with tracheal compression symptoms which had lasted for about 6 weeks. She had taken no medication prior to the occurrence of the thyroid swelling. The patient appeared euthyroid with a very hard nodular enlargement of the left lobe of the thyroid gland. All thyroid parameters were within normal limits. In order to exclude the possibility of carcinoma a subtotal thyroidectomy was performed. The left lobe was stone hard and malignancy was suspected. No infiltration of the adjacent structures was found. A nodular goiter was seen on the right side. The postoperative course was uneventful but 2 months later the patient developed hypothyroidism which was treated with supplementary medication.

In April 1977 the patient was referred to the hospital because of recurrence of the tumor in the left thyroid gland. This time only a biopsy was performed to exclude malignancy definitely.

In Nov. 1978 the patient was admitted to the Medical Department due to lethargy, anorexia, edema in the lower extremities and face and a weight gain of 8 kg. last night for a fortnight. Serum creatinine was markedly elevated (7.1 mg/100 ml). No proteinuria was found. Hydronephrosis with dilated ureters on both sides was seen on an intravenous pyelography and a retrograde pyelography revealed hydronephrosis was due to obstruction in the lumen of the ureters. The left kidney was shrunken (Fig 4).

The patient was transferred to the Urologic Department Aarhus Kommunehospital where a surgeon revealed RF surrounding the common iliac at the fourth and fifth lumbar level and obstructing ureters. Ureterolysis was done on both sides and ureters were placed intraperitoneally. Postoperative, the patient developed deep venous thrombosis in the left leg. The kidney function became normal by the 10th operative day.

Further laboratory studies: Ever since the first admission the patient had had an elevated ESR ranging from 74 mm/h. Serum protein electrophoresis was performed before the last operation but postoperatively immunoglobulinemia was found with elevated IgG. At that time the following laboratory tests were performed: antinuclear factor, LE cell factor, rheumatoid factor, Rose-Waaler's test and antistreptolysin C. All were slightly below the normal limit.

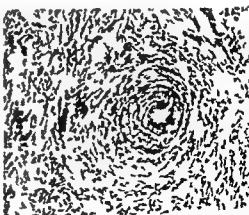


Fig 3 Case 1 The fibrotic thyroid gland contains arteries with lymphocytic infiltration and thickened walls (H & E)



Fig. 4 Case 2. Retrograde pyelography showing medial deviation and constriction of the lower third of the ureters with hydronephrosis and hydroureter.

DISCUSSION

Retroperitoneal fibrosis is characterized by a chronic inflammatory and proliferative fibrous process with various degrees of cellular infiltration of lymphocytes, plasma cells, neutrophilic cells and eosinophils occurring in the loose areolar connective and adipose tissue just beneath the posterior parietal peritoneum of the abdomen and pelvis (8). The ureters are the retroperitoneal structures most frequently involved (11) but cases have been reported in which obstruction of the great vessels, the biliary tracts, the duodenum, the retrosgomdeum and mesenteric vessels have occurred (11). The symptoms and the pathology of RF have been discussed

holog. Microscopic examination of the thyroid gland from the first as well as the second thyroid operation showed severe chronic and subchronic inflammatory changes with lymphocytes, plasma cells and scattered mononuclear cells. A mass of fibrosis was found. The pathological changes extended throughout the thyroid capsule into the adjacent musculature and adipose tissue. No signs of inflammatory reaction were seen (Fig. 5). Examination of a kidney biopsy taken prior to the operation revealed slight interstitial fibrosis. On immunofluorescence microscopy and electron microscopy no glomerular or vacuolar deposits were seen. Microscopic examination of the retroperitoneal tissue showed extensive fibrosis with sparse remnants of fat cells. There was mild mononuclear cell infiltration which was patchy. Small arteries showed thickening of the walls of the intima (Fig. 6).



Fig 5 Case 2 Thyroid gland with severe fibrosis and disappearance of all follicles (H & E)



Fig 6 Case 2 Retroperitoneal fat tissue with fibrosis and patchy lymphocytic infiltration (H & E)

cussed previously (7). The etiology of RF remains an enigma. Koep and Zuidema (11) found an association with methysergide in 12.4% of their cases. Several other etiological factors have been mentioned, such as malignancies, phlebitis in the pelvic veins, retroperitoneal hematoma, aortic aneurysm, chronic intestinal inflammation, sarcoidosis, obstructive lymphangitis, adipose tissue inflammation (Weber-Christian), leakage of urine, toxoplasmosis (11, 16) and α -methyl dopa (6).

The pathological changes resemble those seen in collagen diseases and consequently immunological mechanisms have been considered (8). A few cases have been reported in which RF has been associated with lupus erythematosus, vasculitis (17), amyloidosis (13, 21), immune complex glomerulonephritis (10), Raynaud's phenomenon (17), HLA B 27 antigen (25), as well as deposits of IgG, IgA, IgM on the collagen fibres (6). It has been postulated that methysergide and other drugs act as haptens (8). The good response to steroids in the early stages of RF might support the theory of an immunological hypersensitivity reaction (17).

Riedel's thyroiditis is a unilateral or bilateral proliferative fibrosis which not only involves the thyroid gland but also extends throughout the thyroid capsule into the adjacent structures of the neck and is accompanied by infiltrates of inflammatory cells without giant cells, lymph follicles and granuloma causing destruction of the thyroid parenchyma (26). There are no characteristic biochemical findings associated with the disease. The thyroid function is usually normal and no thyroid antibodies can be demonstrated. Yet the

ESR is generally raised (24). Palpation cannot differentiate Riedel's thyroiditis from carcinoma of the thyroid gland. The etiology is obscure. The usual view, that Riedel's thyroiditis is a late stage of either Hashimoto's or de Quervain's thyroiditis, has now been abandoned (24).

Barrett (2) observed that the pathological changes in Riedel's thyroiditis, RF, pseudotumor of orbit and mediastinal fibrosis are akin to each other. Tubbs (23) published in 1946 a case with coexistent occurrence of mediastinal fibrosis and, in recent years, several cases with simultaneous occurrence of sclerosing fibrosis in a number of different separate organ systems have been described (3, 11, 12, 16, 20). The combination of Riedel's thyroiditis has been reported in other cases (3, 4, 9, 11, 14, 15, 16).

It has been suggested that these apparently disparate fibrotic lesions may be different manifestations of the same disease, called multifocal or systemic idiopathic fibrosclerosis (16). Immunological mechanisms are thought to be etiologically important (9) and it is interesting that Hardt and Hedinger (5) found vascular inflammation in Riedel's thyroiditis similar to Takayasu's arteritis. Familial occurrence has been reported (3) in a case with α 1-antitrypsin deficiency has been published (20). In the present cases arteritis was found in the thyroiditis of case 1 but no biochemical evidence of systemic collagen disease was detected.

The treatment of multifocal idiopathic fibrosclerosis is controversial. All medication should be discontinued. Surgical intervention is necessary when obstruction occurs. Administration of

steroids has given only equivocal results but might be more beneficial in the early stages of the disease (9-17)

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Cortisone-Induced Remission of Hypothyroidism in Schmidt's Syndrome

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STRACT A patient with autoimmune Addison's disease, hypothyroidism and primary gonadal insufficiency is described. Initial treatment with cortisone improved his thyroid function, probably through cortisone suppression of the autoimmune reactions. It was, however, not possible to normalize the thyroid function completely. The decreased testicular function did not improve during the cortisone treatment.

Keywords: Schmidt's syndrome, cortisone induced remission.

Acta Med Scand 208 125 1980

Association between Addison's disease and various endocrine disorders has been established since Schmidt (14) described two patients with non-neoplastic Addison's disease and chronic lymphocytic thyroiditis proved at autopsy. Since then several investigators (1, 2, 6, 8, 10, 15) have described an increased incidence of diabetes mellitus, thyroid disorders, pernicious anaemia, idiopathic hypoparathyroidism and primary hypogonadism in patients with idiopathic Addison's disease. Occurrence of organ-specific antibodies is a characteristic feature of these disorders.

In the following we describe a patient with multiple endocrine insufficiency of autoimmune origin in whom the hypothyroidism remitted during cortisone replacement therapy.

CASE REPORT

The patient was born in 1947 without any family history of endocrine disorders. At the age of 17 he became slightly obese. Several times he consulted a general practitioner. In Oct. 1974 found a decreased S-thyroxine value of 12 nmol/l (normal level >61). No treatment was instituted. In the spring of 1975 the patient went for a holiday to Italy where he was admitted to a hospital because of severe vomiting and abdominal pain. Apart from this he received no treatment. After his return he was

admitted to our hospital. On admission he felt completely well.

At examination pigmentation of oral mucosa was present. Apart from this the physical examination gave normal results. Blood pressure was 110/80 mmHg. S-sodium 120-128 mEq/l, S-potassium 5.7-4.8 mEq/l, 24-hour excretion of 17-ketosteroids 1.0-1.8 mg (normal range 0.7-1.9 mg). No significant increment following stimulation with 1 mg Synacthen® i.m. daily for 3 days. Plasma cortisol was less than 110 nmol/l (normal range 240-810). No increment of plasma cortisol was registered after stimulation with 0.25 mg Synacthen® given as a single i.m. injection. After stimulation with 1 mg Synacthen® daily for 3 days plasma cortisol increased to subnormal values (116 and 154 nmol/l). TSH was 340 mU/l (normal range <19). Total S-thyroxine was 19 nmol/l (normal range 60-140). T₃RIA was 1.21 nmol/l (normal range 1.14-2.30). LH was within the normal range at repeated examinations. FSH was constantly increased 31, 35 and 32 mU/ml (normal range 5-20). S-testosterone was 472-713 µg/100 ml (normal range 299-791). In glucose tolerance test including insulin measurements was within the normal range. Organ-specific antibodies (see Table 1). X-rays of sella turcica, thorax and adrenals (including tomography) were normal. Ophthalmoscopy and vision field were normal. Histocompatibility (HLA) antigen type was HLA A1, 29, B8, 4, A2 (TT).

The patient was treated initially with cortisone acetate and later with fludrocortisone acetate as shown in Fig. 1. The clinical remission was immediate and impressive. S-sodium and S-potassium normalized quickly. The course of the thyroid function tests is shown in Fig. 1. In Jan. 1976 the patient felt increasing fatigue but had no other complaints. The dose of cortisone was increased but as this had no effect on the patient's condition and as the thyroid function was not within the normal range, therapy with levothyroxine was started. The patient has since then been in clinical remission. The patient, a single man, has on no occasion made any spontaneous complaints about his sexual function.

DISCUSSION

The examinations demonstrate that the patient suffers from both hypothyroidism and Addison's disease (Schmidt's syndrome). The increased FSH

Table 1 Organ specific autoantibodies before and after initiation of cortisone replacement therapy

	Before treatment	After treatment
Thyroglobulin antibody CA 1	0	0
Second colloid antibody CA 2	+	0
Microsomal thyroid antibody	+	++
Adrenocortical antibody		+
Parietal cell antibody		II
Islet cell antibody		0

values indicate that he also has insufficient gonadal function (7-9)

Idiopathic Addison's disease is considered an autoimmune disorder with occurrence of antibodies against the adrenal cortex (1, 5, 6, 11) in contrast to tuberculous hypoadrenalism (5, 11)

Primary hypothyroidism is likewise considered an autoimmune disease possibly the final stage in Hashimoto's thyroiditis (4, 12). The autoimmune pathogenesis for two of the patient's endocrine diseases suggests that the same mechanism may be responsible for the third, the insufficient gonadal function.

The patient's thyroid function improved after the start of cortisone replacement therapy. The TSH values decreased significantly and S-thyroxine increased to subnormal level. Significant and lasting improvement of thyroid function in four patients with Schmidt's syndrome substituted solely with adrenal steroids has previously been described (3). It has been suggested that the underlying mechanism is a suppression of the destructive process in the thyroid gland by adrenal steroids. Adrenal steroids exert an effect on the pituitary function. Re et al (13) have demonstrated that glucocorticoids in physiological and pharmacological doses suppress the S-TSH level in euthyroid and hypothyroid subjects through an effect on the pituitary gland and that S-thyroxine is not altered by the glucocorticoids. The significant and lasting decrease in S-TSH and the synchronous increase in S-thyroxine in our patient clearly cannot be ascribed to a glucocorticoid effect on the pituitary gland but indicate that a regeneration of the thyroid function has taken place.

Ghanb et al (3) assume that the restitution of the thyroid function is due to a suppressing effect of cortisone on the destructive processes in the thyroid gland. In our patient the CA 2 antibodies

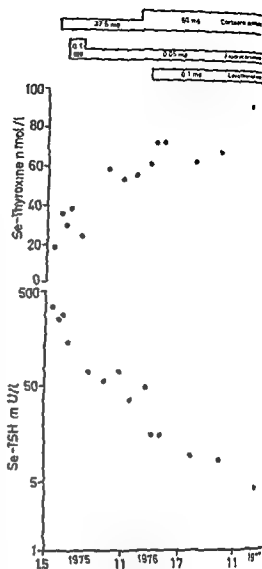


Fig. 1 Total S-thyroxine (normal range 60-140 nmol/l) and S-TSH (normal level ≈ 1.9 mU/l) plotted against

disappeared synchronously with the improvement of the thyroid function. This might support the hypothesis that cortisone in physiological doses suppresses the immunological processes responsible for the impaired thyroid function. It is also ever remarkable that the other autoantibodies never changed in our patient and that the testis dysfunction was not improved.

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Symptomatic Diabetes Mellitus Cured by Potassium and Withdrawal of Polythiazide in a Hypokalemic Hypertensive Woman

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STRACT A case of symptomatic diabetes mellitus in a 65 year old woman with 14 years of thiazide treatment and hypokalemia is described. The condition was cured by potassium administration and withdrawal of the thiazide diuretic.

words: diabetes mellitus diuretics potassium deficiency

Acta Med Scand 208 129 1980

Hyperuricemia and glucose intolerance caused by thiazide diuretics and probably mediated by hypokalemia has been observed for several years (1-9, 11, 12, 13, 14, 18, 19, 20). Most often this hyperuricemia is moderate. The present case history is therefore of some interest as it concerns a mild symptomatic case of diabetes mellitus cured by withdrawal of polythiazide and administration of potassium in a hypokalemic hypertensive patient.

CASE HISTORY

A 65-year-old woman was admitted to hospital because of diabetes. She had no family history of diabetes or hypertension. Cholecystectomy at age 44. Hysterectomy for leiomyoma uteri at age 50. Subtotal thyroidectomy for toxic nodular goiter at age 51. When she was 52 years old a general practitioner detected arterial hypertension. The arterial values of blood pressure at that examination are not available. The patient had never suffered from dyspnea, chest pain or swollen legs. Polythiazide (Renescan[®]) 100 mg once a day was prescribed. One month before admission to our hospital she noticed thirst and frequent urination with large amounts of urine. Diabetes mellitus was suspected and the patient was hospitalized.

On admission examination she was dehydrated. There was tachypnea, cyanosis or signs of abnormal thyroid function. The cardiac and pulmonary auscultatory findings were normal. The blood pressure was 165/95 mmHg, body weight 74.5 kg, Hb 168 g/l, ESR 4 mm/h. ECG showed a complex in leads V₁-V₂ indicating transmural anterior

damage of the myocardium of unknown age. Furthermore, distinct U waves were found. The fasting blood sugar was 24.5 mmol/l and 258 mmol glucose was excreted during 24 hours in 2 l urine. Trace amounts of ketone bodies were found in the urine. Serum concentration of sodium was 142 mmol/l, potassium 2.1 mmol/l and chloride 94 mmol/l indicating grave alkalotic hypokalemia. Serum creatinine was 71 µmol/l. Albustix[®] was negative.

In view of the possible connection between thiazide induced hypokalemia and diabetes mellitus, treatment was started with seponation of polythiazide and oral administration of 4 g potassium chloride b.i.d. Diabetes diet was prescribed during five days whereafter the patient was transferred to an ordinary diet. The fasting blood glucose level progressively fell during a period of 12 days after which it was 5.8 mmol/l (Fig 1). Normal value in this age group is 4.6 ± 0.5 mmol/l (mean ± S.D.). The glucosuria gradually disappeared and could not be demonstrated after the seventh hospital day. Trace amounts of ketone bodies initially found in urine had disappeared after two days. The body weight increased from 74.5 to 76.2 kg during the first two weeks in hospital. Serum potassium rose to 3.9 and serum chloride to 106 mmol/l during the same period.

After two weeks the patient felt quite well and left the hospital with potassium chloride 1 g t.i.d. as the only prescription. Two months later she felt well and had no symptoms of diabetes mellitus. The fasting serum glucose level was then 4.9 mmol/l which is within the normal range. The peroral glucose tolerance test showed a maximum of 10.7 mmol/l 60 min after administration of 1 g glucose/kg b.wt., a value also within the normal limits. Serum sodium was 141 mmol/l, potassium 4.0 mmol/l and chloride 106 mmol/l. She weighed 75 kg and had a blood pressure of 150/90 mmHg. The patient had a slight angina pectoris and nitroglycerin was prescribed. Otherwise she had no drug or diet therapy.

DISCUSSION

Both well controlled diabetes patients and patients without prior evidence of carbohydrate intolerance have developed hyperglycemia and glucosuria after

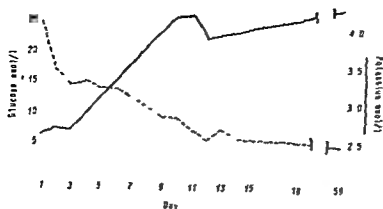


Fig 1 Serum potassium (---) blood glucose (—) after polythiazide withdrawal and institution of treatment with potassium chloride 1 g t.i.d.

treatment with thiazide diuretics for periods varying from one day to several years (2, 6, 9, 11–14, 18–20). The topic has been extensively reviewed by Bengtsson (2) who also found contradictory reports in the literature. In particular longer acting thiazide diuretics like polythiazide have been reported to give an adverse effect on glucose tolerance (5). Previous evidence of this adverse thiazide effect has been casuistic or founded on patient series without randomized controlled trials so that it has been difficult to differentiate between spontaneous and drug induced changes in glucose metabolism. A reversibility of the impairment of carbohydrate tolerance on seponation of diuretics has suggested drug effects in some cases (2, 12). Recently Amery et al (1) have strengthened the concept of an adverse effect of thiazide diuretics on carbohydrate tolerance in a double blind randomized controlled trial of 119 elderly hypertensive patients. These authors found that unlike the findings in a placebo group the average fasting blood sugar rose and the glucose tolerance during an oral glucose load deteriorated progressively at controls 1, 2 and 3 years after treatment with 25 mg hydrochlorothiazide and 50 mg triamterene. During the three years the average rise in fasting blood sugar was 14 mg/100 ml (1 mmol/l). The changes in glucose tolerance in this series must be characterized as mild and no cases of diabetes developed. The deterioration in glucose tolerance did not appear to be limited to a few patients but occurred "across the board". This suggests that the risk of inducing clinical diabetes by means of thiazide treatment is largest in persons with borderline diabetes. It is therefore important to observe that hypertension which is a main indication for thiazide therapy is

associated with diabetes irrespective of intensive treatment (7).

The mechanism behind the effect of blood glucose tolerance is not entirely clear but lines of evidence point to a close connection between thiazide induced potassium loss paired glucose tolerance. It has been known long time that the potassium ion influence carbohydrate metabolism in several ways so as an activator of enzymatic reactions: glucogen formation (10), Sagild et al (17), marked decrease in carbohydrate tolerance. potassium depletion was induced in normal oral ion-exchange resin. This finding is in harmony with results of potassium depletion (8) as well as with the observation of Rapin Hurd (16) that glucose tolerance improved with potassium supplementation in thiazide treated patients with hypokalemia. The same effect of potassium supplementation has been observed in who are hypokalemic from primary or secondary hyperaldosteronism (15). The study by Amery (1) also demonstrated a close parallelism between the lowering of serum potassium and deterioration of carbohydrate tolerance. On the other hand studies have failed to demonstrate any association between hypokalemia and pathological glucose tolerance during treatment with thiazide diuretics (14).

The patient presented here shows a reversibility of symptomatic diabetes associated with thiazide treatment and hypokalemia. This suggests that withdrawal of thiazide diuretic in combination with correction of potassium deficiency possibly may cure symptomatic diabetes mellitus. It is previously known that potassium supplement-

well as withdrawal of the azide diuretics may improve glucose tolerance in both diabetic and non-diabetic subjects (13-15, 16) but the present patient seems to be remarkable in view of the high degree of improvement. In a survey of the literature I find only one report with a comparable improvement of diabetes on withdrawal of diuretic treatment (17) and that report contained no data on potassium levels.

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A Case of Hypocalcemia, Heart Failure and Exceptional Repolarization Disturbances

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TRACT Little attention has been given to the question whether clinical heart failure can be a manifestation of hypocalcemia. A patient with hypoparathyroidism and heart failure prompted us to analyse reports on this subject. The conclusion was that associated with an underlying myocardial disease, hypocalcemia may be a rare contributing factor to heart failure. Hypocalcemic heart failure without underlying heart disease has been suggested as a cause of tension in two special situations in which a fall of serum ionized calcium is induced: massive transfusions of citrated blood and rapid correction of uremic acidosis. In addition to hypocalcemia and heart failure, our patient had exceptional repolarization disturbances: rate-dependent variations of T wave amplitudes during sinus arrhythmia, unexpected prolongations of the Q-T interval and attacks of ventricular tachycardia.

ords: hypocalcemia, heart failure, repolarization disturbances, tachycardia.

Med Scand 208 133 1980

We report a patient who had suffered from severe thyrotoxicosis for several months. Six months after thyroid resection she was admitted to hospital with hypocalcemic pulmonary edema and circulatory shock. The clinical signs of heart failure were cured by calcium administration. The subsequent course was complicated by repolarization disturbances and arrhythmias.

CASE REPORT

A 38-year-old girl was admitted to hospital in severe thyrotoxicosis after having suffered from palpitations and tachycardia for several months. On admission a supraventricular tachycardia of 180/min was recorded. PBI was 15.9 µU/ml. There was nothing exceptional in her family or past history. An innocent systolic murmur had been found at routine medical check-up of school children at the

age of 14. Chest roentgenogram and ECG (Fig. 1A) were normal at that time.

She was treated with carbimazole, propranolol and preoperatively with potassium iodide. The preoperative chest roentgenogram and ECG were interpreted as normal. On reanalysis of the chest roentgenogram the heart was, however, found to be slightly enlarged (Fig. 2A).

She underwent an ordinary thyroid resection and was discharged in good condition. At home she began to feel progressive muscle twitches and weakness on exertion but did not consult a doctor. Six weeks after operation she became powerless and was readmitted.

A pale, exhausted and dyspneic girl was seen on admission. Her physique was normal, weight 62 kg, height 164 cm. She was in circulatory shock; the arterial pulse was feeble and the blood pressure could not be recorded; the skin was cold and clammy. Moist rales were heard over both lungs. Neck veins were not distended and there was no peripheral edema. Chvostek's sign was strongly positive. ECG displayed sinus tachycardia of 125/min, left atrial strain and a markedly prolonged Q-T interval. The observed Q-T interval (Q-T_o) was 0.38 sec, the normal Q-T interval (Q-T_n) for the heart rate being 0.27 sec (Fig. 1B). Chest roentgenogram showed alveolar edema and an enlarged heart (Fig. 2B). Because of the strong clinical suspicion of postoperative hypoparathyroidism, administration of calcium gluconate was started immediately. Determined on admission: serum calcium 4.1 mg/100 ml and serum phosphate 8.5 mg/100 ml confirmed the clinical diagnosis. After i.v. administration of 20 ml of 10% calcium gluconate as an emergency treatment, blood pressure rose to 90 mmHg. The central venous pressure was 12 cm H₂O. Electrolytes were normal. During the following 12 hours the patient received a total of 60 ml calcium gluconate as the only treatment (apart from oxygen and i.v. infusion of 5% glucose with potassium, but neither digitalis nor vasopressors or diuretics). She improved remarkably; the blood pressure rose to 105/90 mmHg and the chest roentgenogram showed clear lung fields. Echocardiogram excluded pericardial effusion as the cause of the heart enlargement. Oral substitution of calcium and dihydrotachysterol was instituted.

During the following two weeks, transient periods of marked sinus arrhythmia developed with rate-dependent

Abbreviations: Q-T_o observed Q-T interval, Q-T_n normal Q-T interval.

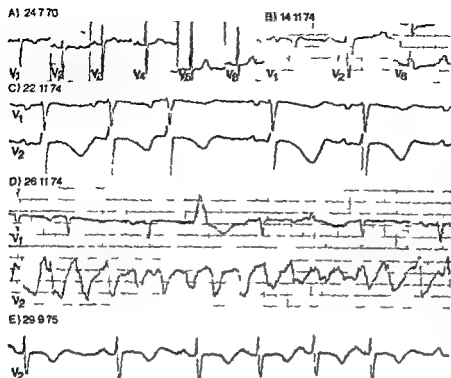


Fig. 1. Serial ECG (sec) (A) Normal (notched T waves) preoperatively (B) prolonged Q-T interval; atrial strain on admission (C) Marked sinus arrhythmia with rate-dependent variation (D) Sinus arrhythmia and late ventricular extrasystoles (V1) preceding a ventricular tachycardia (V2 chosen to show changing direction complex) (E) Sinus rhythm without T waves after recovery

variation of T wave amplitudes and hysteresis of Q-T interval (Fig. 1C). Accompanying these periods the patient had recurrent attacks of ventricular tachycardia. The ventricular tachycardias were heralded by subjective feeling of hot waves going over the body and bursts of late ventricular extrasystoles apparently provoked by long R-R intervals during sinus arrhythmia (Fig. 1D). During intervening normal sinus rhythm unexpected varying prolongations of the Q-T interval were noted (Q-T₀ up to 50% longer than Q-T₁). The Q-T interval was most markedly prolonged during days with several episodes of ventricular tachycardia. This fluctuation of the Q-T interval did not parallel the level of serum calcium: sudden Q-T prolongations occurred during stable normocalcemia. No explanation of these disturbances was found at a thorough examination. All routine laboratory tests, e.g. serum magnesium and proteins, were normal.

The patient gave a strong clinical impression of an enhanced sympathetic tone with her apprehensive appearance and dilated pupils, but urinary excretion of epinephrine, norepinephrine, metanephrines and vanillyl mandelic acid, as well as electroencephalogram, were normal. The following drugs proved ineffective: lidocaine with or without overdrive ventricular pacing, practolol, procainamide and quinidine. Mostly the tachycardias disappeared spontaneously, tens of DC shocks were also needed. After starting diphenylhydantoin treatment 300 mg daily, the arrhythmias and Q-T prolongations discontinued abruptly and completely and did not recur. The patient was discharged in good condition on 2 g calcium, 0.4 mg dihydrotachysterol and 300 mg diphenylhydantoin daily.

A check up one year later revealed that the heart size

had reverted to normal (Fig. 2C). Signs of autonomic lability were found when the patient from the supine to upright position. In the supine the blood pressure was 146/88 mmHg; it displayed sinus rate of 63/min, Q-T equal to C, a normal T wave polarity. Standing up caused the blood pressure to 110/90 mmHg and marked changes in the ECG: sinus arrhythmia with a rate 125/min and hysteresis of Q-T interval, inverted waves, but no rate-dependent amplitude variation and high voltage P waves (Fig. 1E). T arrhythmia could not be provoked after pre-treatment with propranolol 5 mg i.v.

The physical performance of the patient was good and she tolerated heart rates of over 180/min on an ergometer without subjective symptoms or changes in the ECG.

Administration of diphenylhydantoin was done without adverse consequences. An attempt was made to withdraw calcium and dihydrotachysterol but she could not do without substitution. When seen three years later she was still feeling completely healthy and had no heart disturbances.

DISCUSSION

Though calcium plays a key role in the contractile process in myocardial cells, the clinical symptoms of hypocalcemia usually derive from an increased excitability of the nervous system and hypocalcemia is rarely considered as a cause of heart

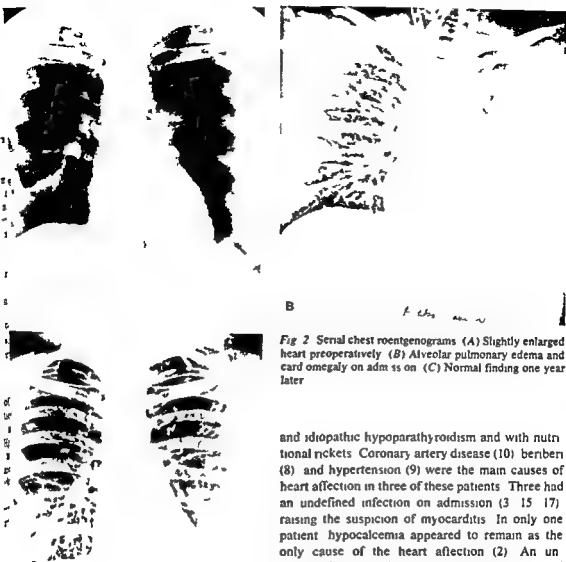


Fig 2 Serial chest roentgenograms (A) Slightly enlarged heart preoperatively (B) Alveolar pulmonary edema and cardiomegaly on admission (C) Normal finding one year later

and idiopathic hypoparathyroidism and with nutritional rickets. Coronary artery disease (10), beriberi (8) and hypertension (9) were the main causes of heart affection in three of these patients. Three had an undefined infection on admission (3, 15, 17) raising the suspicion of myocarditis. In only one patient hypocalcemia appeared to remain as the only cause of the heart affection (2). An unexplained symptomless cardiomegaly and return of heart size to normal with restoration of normocalcemia has been reported twice in hypoparathyroidism (1, 4).

Heart failure has not been ascribed to hypocalcemia in chronic untreated uremia. The explanation might be uremic acidosis keeping the serum level of ionized calcium low. A rapid fall in ionized calcium during vigorous treatment of advanced uremia (5) as well as massive transfusions of citrated blood (11, 20) may lead to hypotension, the suggested pathogenesis being a reduction of the systemic resistance and/or decrease in contractile force.

In our patient pulmonary edema and circulatory shock denoting left ventricular failure were fully treated with calcium alone. Even so, h

In neonatal hypocalcemia symptoms have partly attributed to heart failure (7, 18). How the correct interpretation of the clinical signs difficult since edema in neonatal hypocalcemia is not necessarily a sign of heart failure but may result from renal effects of hypocalcemia itself (18). Hypocalcemia causing or aggravating heart failure, an apparent response to calcium therapy has been reported in few patients with postoperative

cemia alone could hardly have caused her clinical condition. The condition was acquired one year after this episode; there were no signs of a heart disease. The long and fulminant history of thyrotoxicosis with supraventricular tachycardia of 180/min on the first admission and the radiological evidence of heart enlargement preoperatively suggested that she had an underlying thyrotoxic heart disease. This and the previous case histories suggest that if associated with an underlying myocardial disease, hypocalcemia may be a rare contributing factor to heart failure. However in special situations a sudden fall in ionized calcium may be enough for making the heart fail.

The repolarization disturbances and arrhythmias need commenting. Normally, T wave amplitude is stable during sinus arrhythmia (13) as it was in our patient after recovery (Fig. 1E) but during the acute phase the amplitude varied with the length of the preceding diastole (Fig. 1C). This phenomenon has been previously described in atrial fibrillation as a sign of heart disease, probably resulting from the mechanical effect of varying diastolic filling on repolarization of diseased myocardium (16). During the attacks of ventricular tachycardia the direction of QRS complex undulated (Fig. 1D) resembling torsade de pointes, an atypical ventricular tachycardia which occurs especially in patients with prolonged Q-T interval due to asynchrony of repolarization (11). However repolarization remains synchronized in hypocalcemia and there should be no predisposition to torsade de pointes. Furthermore, the ventricular tachycardias and Q-T prolongations were unrelated to the serum calcium level. Several features of our patient (sudden prolongations of Q-T interval, feeling of hot waves preceding the ventricular tachycardias, periods of sinus arrhythmia) were typical of the hereditary Romano-Ward syndrome (12, 19) in which changes in autonomic tone with inappropriate cardiac sympathetic discharges are supposed to induce asynchronous repolarization and precipitate ventricular arrhythmias. Suggestively, our patient exhibited signs of a marked constitutional autonomic lability already reflected by the notched T waves (14) in her childhood ECG (Fig. 1A). The effects of this autonomic lability on diseased myocardium could have been of significance.

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Osteosclerotic Myeloma with Polyneuropathy

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ABSTRACT Five cases of histologically verified plasma cell myeloma in sclerotic skeletal foci and neuropathy are reported. Thirty similar cases collected from the literature. They illustrate a new form of plasma cell neoplasia. The characteristic features are osteosclerosis, polyneuropathy, and polyradiculitis, approximately normal globulin concentration, bone marrow smears and absence of Jones proteinuria. Slow progression of the disease is a possible additional feature. It is hypothesized that autoimmune mechanisms are involved in the pathogenesis. This hypothesis is based on the observation of circulating immune complexes, the Waaler reaction and relative increase in the number of circulating B-lymphocytes.

Key words: myeloma, osteosclerosis, polyneuropathy, immunology, neurophysiology, pathogenesis.
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Plasmacytoma and multiple myeloma are caused by clonal proliferation of plasma cells. In most cases the tumour cells belong to the same clone and therefore produce uniform immune protein (protein). The classical radiological features are multiple minute foci giving the bone an osteosclerotic appearance or larger osteolytic defects. These are often circular and well defined with no surrounding sclerosis. Treatment of such lesions by radiation or cytotoxic agents may result in osteosclerosis. However, rare cases with osteosclerosis prior to treatment have been reported and a remarkable number of them have also had radiological changes consistent with polyneuropathy.

We present five additional cases and discuss the relationship between the primary osteosclerosis, polyneuropathy and other findings characteristic of this syndrome.

CASE REPORTS

Case 1

Female, born in 1914. The first symptoms appeared in 1966 with restless legs, general weakness and difficulty in walking. Within a few months she became totally unable to walk and lost a great deal of weight.

Neurological examination in Jan. 1967 showed a sensorimotor polyneuropathy with pareses, hypoesthesia and hypoaesthesia and absence of deep tendon reflexes in both legs. The arms were not clinically affected except for weak deep tendon reflexes. Roentgenogram revealed multiple osteosclerotic foci throughout the skeleton. Histologically the foci contained atypical plasma cell infiltrates in cavities within sclerotic bone with thickened lamellae with many cement lines indicating period of growth. Between the normal bone and the plasma cell infiltrates there were concentrations of mature lymphocytes with lymph follicles. The plasma cell infiltrates contained a homogeneous eosinophilic substance, probably a result of globulin production. Hb was 151 g/100 ml, ESR 3 mm/hour. Bone marrow smears showed less than 2% plasma cells. Serum contained monoclonal IgG. Lambda in low concentration. EMG recorded in the right arm and both legs showed a peripheral neurogenic lesion. The motor conduct on velocity was slightly reduced in the right ulnar nerve and could not be recorded in the left extremities because of atrophic musculature. Spinal fluid protein concentration was 161 mg/100 ml. The fluid did not contain cells. Liver and kidney function tests were normal.

The condition was considered to be a myeloma and treatment was started with melphalan and prednisone. Melphalan was discontinued after a few months. The neurological symptoms improved slightly with prednisone treatment until 1970. Prednisone was discontinued in 1975.

In 1976 roentgenograms showed considerable reduction of the osteosclerotic foci and nearly normal bone structure. Hb was 133 g/100 ml and ESR 4 mm/h. No rheumatoid factor or immune complexes were found in the serum, neither were immune complexes found in nerve and muscle biopsies. There were 15% B lymphocytes and 37% T-lymphocytes in the blood. Bone marrow, liver and kidney function tests were normal. The clinical findings have later remained virtually unaltered.

Case 2

Male born in 1905 (previously published by Smith Meyer (54)). The diagnosis of myeloma based on biopsy from a sclerotic rib lesion was established in 1956. The patient was observed without any treatment until 1964 when a laminectomy had to be performed because of tumour pressure on the spinal cord producing paresis. Histological examination of the tumour showed highly cellular tissue with large and pleomorphic plasma cells. Hb was 1.7 g/100 ml, ESR 14 mm/h.

In the following year the patient began to complain of pain in hands and feet. Roentgenograms showed sclerotic lesions in the upper femur and iliac bone on the right side and 4th lumbar vertebra. Hb was 12.1 g/100 ml, ESR 70 mm/h. There were 10% plasma cells, some of them atypical in the sternal bone marrow smears. He was treated with melphalan for a short time. In 1966 the pain in hands and feet had increased and was especially localized to the small joints. Neurological examination showed sensorimotor polyneuropathy in arms and legs with distal pareses, hypoesthesia and absent deep tendon reflexes. The legs were cyanotic and showed reduced arterial pulsation. Roentgenograms showed sclerosis in another rib. Hb was 15.1 g/100 ml and ESR 11 mm/h. Serum protein electrophoresis showed a small pathological fraction between the gamma and beta fields. Immune electrophoresis showed monoclonal IgA kappa in moderate concentration. EMG showed signs of a peripheral neuropathy with motor nerve conduction velocity in the median nerve reduced to 20 m/sec. Throughout 1967 the general condition worsened and the patient complained of increased rheumatoid pain in knees, ankles and feet. Acrocyanosis increased in hands and feet. The neurological symptoms also increased. A tibial muscle biopsy showed mild cell atrophy. Hb was 6.9 g/100 ml and ESR 80 mm/h. In the sternal bone marrow smear there were 5-10% plasma cells.

The patient died in 1968 of renal failure. Autopsy disclosed multiple myeloma infiltration in the skeleton. The infiltrations in the lumbar vertebra were surrounded by bone showing several cement lines but no sclerosis. The bone around the infiltrations in the affected ribs was sclerotic. However, the cell types in these infiltrates were of uncertain nature. Plasma cell infiltrates suggesting myeloma infiltrates were found in enlarged lymph nodes in the mediastinum. The kidneys showed signs of chronic pyelonephritis and the liver and spleen were congested. Amyloidosis could not be found.

Case 3

Male born in 1916. After some years with periodic low back pain the patient was hospitalized in 1965 for investigation of pain and weakness in legs and hands. The musculature was slightly atrophic. Roentgenograms of the spine were normal. The spinal fluid protein was 163 mg/100 ml with normal pressure. The condition was considered to be a form of generalized myalgia and was treated with physiotherapy.

The patient was readmitted in 1966. Roentgenograms now revealed polycyclic osteolytic destruction in the sacrum with surrounding sclerosis. Biopsy showed myeloma. The sternal bone marrow smear was normal. Elec-

trophoresis showed slight increase of β -globulin in the serum. The patient was treated with a dose of 3000 R.

In 1970 the patient developed sensorimotor neuropathy with symmetrical paresis of all four limbs, most marked in the legs and with absent deep reflexes. There were hypoesthesia and hypoaesthesia in the legs but no sensory changes in the arms. Roentgenograms now showed increased destruction of the lumbar vertebrae. Hb was 15.0 g/100 ml, ESR 15 mm/h. There were less than 5% plasma cells in the sternal marrow smear. Electrophoresis showed increased protein component between the gamma fields. Immune electrophoresis showed two components of IgA type (later examination showed component IgA lambda). EMG and nerve conduction studies were not carried out.

After treatment with cyclophosphamide the sclerosis increased, the electrophoresis curve normal and the pareses improved. The improvement followed after replacement of cyclophosphamide with melphalan.

Reexamination in 1977 still showed bilateral paresis but there were normal deep tendon reflexes. Roentgenograms showed unchanged sclerosis. Hb was 15.0 g/100 ml, ESR 4 mm/h. Liver and kidney tests were normal. Electrophoresis and immune electrophoresis were normal. Rheumatoid factor, clear factor and an ANA were not found. Must from the leg showed atrophic fibres without amyloid infiltration. There was no amyloid in the biopsy. Lymph node biopsy showed no amyloid. The patient has been in full remission for 10 years.

Case 4

A female born in 1913 had for several years from low back pain and had a transient right paralytic in 1960. In early 1974 she developed paresthesia in legs and arms. She had hypoaesthesia, deep tendon reflexes were absent. Ophthalmoscopy showed early papilloedema. Roentgenograms showed multiple dense sclerotic foci in the skeleton. These were seen on earlier films as far back as 1972. Hb was 11.5 g/100 ml, ESR 8 mm/h. No rheumatoid factor was found. Lumbar spinal fluid showed normal pressure, protein 100 mg/100 ml and normal cell count. Immunoelectrophoresis showed monoclonal IgG lambda in low concentration in serum and spinal fluid. EMG showed a peripheral neuropathy in the right leg and arm. Motor conduction velocity in the right ulnar nerve was 21 m/sec. Brain scan, pneumoencephalography, cerebral angiography and myelography were normal.

Progression of the pareses and the sclerosis continued in the spring of 1975 (Fig. 1). Pedal lymphography showed defects of uncertain significance. In multiple lymphography Hb fell to 9.2 g/100 ml and ESR rose to 54 mm/h. Spinal protein rose to 732 mg/100 ml although the count was still normal. A biopsy of a sclerotic condyle showed plasma cell infiltration surrounded by dense infiltrates of lymphocytes adjacent to extensive sclerotic areas of bone with multiple cement lines. The lesion



Fig 1 Case 4 1975 Osteosclerotic skeletal myeloma foci

considered to be a myeloma. A lymph node biopsy demonstrated dense plasma cell infiltration probably of her than neoplastic nature.

Condition stabilized and from the summer 1975 onwards some improvement during chemotherapy with cyclophosphamide and prednisone was noted and also with procarbazine addition. The improvement ceased after a short while and the chemotherapy discontinued. Later in 1975 her hands still showed fractures with paresis. Hb was 9.3 g/100 ml and ESR 100 mm/h. Serum creatinine was slightly elevated and there was slight proteinuria without any sign of Bence Jones protein. The spleen was enlarged on isotope scans. Muscle biopsy showed neurogenic atrophy. The rectal mucosa histologically normal. The patient's serum contained rheumatoid factor (Waaler titer 256, Latex ++), and there were circulating immune complexes in moderate amounts. Of the blood lymphocytes 49% were B and 36% T lymphocytes. Immune complex deposits were not found in nerve and muscle biopsies.

The general condition deteriorated during the spring of 1976 and the neurological signs progressed. Roentgenograms showed increased skeletal involvement, partly osteolytic, partly osteosclerotic. In early 1978 the patient developed edema, lung infiltrates and bilateral pleural effusion containing atypical plasma cells. The sclerotic foci in the skull disappeared. Finally the Hb fell and the patient died in 1978.

Case 5

Male born in 1931. Throughout many years he had had attacks of drolthiasis and had since 1968 been treated for hypertension. In late 1975 he developed periorbital edema and edema of the hands and feet. In 1976 he progressively developed a polyneuropathy

with distal and symmetrical pareses in the legs. The deep tendon reflexes disappeared in both legs and arms. There was no sensory loss. In the spring of 1976 he developed joint pains and morning stiffness in the hands. The pareses progressed and hypoesthesia developed first in the legs and later in the arms. During the night he sweated and had chills. The small finger joints became swollen and the feet enlarged. Roentgenograms showed enlargement of the heart and pulmonary congestion. A sclerotic tumour was apparent on X ray of the left half of the pelvis. On earlier films the tumour could be seen as far back as 1970. Biopsy showed plasma cell myeloma. A homogeneous eosinophilic substance possibly local globulin was found in the sinusoids in the tumour tissue. Biopsy of the inguinal lymph nodes and from the rectal mucosa showed dense plasma cell infiltration. Hb was 14.7 later 11.9 g/100 ml. ESR was 41 later 46 mm/h. Serum electrophoresis showed elevated β -globulin. Immune electrophoresis revealed a low concentration of monoclonal IgA with indefinite light chains. Tests for rheumatoid factor were positive (Waaler titer 16, Latex ++++). Bone marrow smears were normal. The liver and spleen were enlarged on isotope scans. Lymphography showed multiple defects in the lymph nodes as seen in lymphoma. Serum creatinine was 1.8 later 2.9 mg/100 ml. There was slight proteinuria without Bence Jones protein. Lumbar spinal fluid showed a protein concentration of 99 later 146 mg/100 ml. EMG showed a peripheral neurogenic changes. The motor nerve conduction velocity was 17 m/sec in the peroneal nerve. The tumour area in the pelvic bones and the iliac and lumbar lymph nodes were treated with external radiotherapy to a dose of 40 Gy. In addition combination chemotherapy was started with cyclophosphamide, procarbazine and prednisone. His congestive heart failure was treated with digoxin and diuretics.

Case 2

Male born in 1905 (previously published by Smith Meyer (54)). The diagnosis of myeloma based on biopsy from a sclerotic rib lesion was established in 1956. The patient was observed without any treatment until 1964 when a laminectomy had to be performed because of tumour pressure on the spinal cord producing paresis. Histological examination of the tumour showed highly cellular tissue with large and pleomorphic plasma cells. Hb was 12 g/100 ml, ESR 14 mm/h.

In the following year the patient began to complain of pain in hands and feet. Roentgenograms showed sclerotic lesions in the upper femur and iliac bone on the right side and 4th lumbar vertebra. Hb was 12.1 g/100 ml, ESR 20 mm/h. There were 10% plasma cells, some of them atypical in the sternal bone marrow smears. He was treated with melphalan for a short time. In 1966 the pain in hands and feet had increased and was especially localized to the small joints. Neurological examination showed sensorimotor polyneuropathy in arms and legs with distal pareses, hypoesthesia and absent deep tendon reflexes. The legs were cyanotic and showed reduced arterial pulsation. Roentgenograms showed sclerosis in another rib. Hb was 11.1 g/100 ml and ESR 11 mm/h. Serum protein electrophoresis showed a small pathological fraction between the gamma and beta fields. Immune electrophoresis showed monoclonal IgA kappa in moderate concentration. EMG showed signs of a peripheral neurogenic lesion with motor nerve conduction velocity in the median nerve reduced to 20 m/sec. Throughout 1967 the general condition worsened and the patient complained of increased rheumatoid pain in knees, ankles and feet. Acrocyanosis increased in hands and feet. The neurological symptoms also increased. A tibial muscle biopsy showed muscle cell atrophy. Hb was 6.9 g/100 ml and ESR 80 mm/h. In the sternal bone marrow smear there were 5–10% plasma cells.

The patient died in 1968 of renal failure. Autopsy disclosed multiple myeloma infiltration in the skeleton. The infiltrations in the lumbar vertebra were surrounded by bone showing several cement lines, but no sclerosis. The bone around the infiltrations in the affected ribs was sclerotic. However, the cell types in these infiltrates were of uncertain nature. Plasma cell infiltrates suggesting myeloma infiltrates were found in enlarged lymph nodes in the mediastinum. The kidneys showed signs of chronic pyelonephritis and the liver and spleen were congested. Amyloidosis could not be found.

Case 3

Male born in 1926. After some years with periodic low back pain the patient was hospitalized in 1965 for investigation of pain and weakness in legs and hands. The musculature was slightly atrophic. Roentgenograms of the spine were normal. The spinal fluid protein was 163 mg/100 ml with normal pressure. The condition was considered to be a form of generalized myalgia and was treated with physiotherapy.

The patient was readmitted in 1966. Roentgenograms now revealed polycyclic osteolytic destruction in the sacrum with surrounding sclerosis. Biopsy showed myeloma. The sternal bone marrow smear was normal. Elec-

trophoresis showed slight increase of β -globulin. Sacrum was irradiated with a dose of 3000 R.

In 1970 the patient developed sensorimotor neuropathy with symmetrical paresis of distal most marked in the legs and with absent deep reflexes. There were hypoesthesia and hypesthesia in the legs but no sensory changes in the arms. Roentgenograms now showed increased destruction of the ilium. Rounding bone was sclerotic. Hb was 15.0 g/100 ml, ESR 15 mm/h. There were less than 5% plasma cells in the sternal marrow smear. Electrophoresis showed increased protein component between the gamma fields. Immune electrophoresis showed two components of IgA type (later examination showed component IgA lambda). EMG and nerve velocity studies were not carried out.

After treatment with cyclophosphamide the sclerosis increased, the electrophoresis curve normal and the pareses improved. The patient died after replacement of cyclophosphamide with melphalan.

Reexamination in 1977 still showed bilateral but there were normal deep tendon reflexes in the legs. Roentgenograms showed unchanged sclerosis. Hb was 15.0 g/100 ml, ESR 4 mm/h. Liver function tests were normal. Electrophoresis and immune electrophoresis were normal. Rheumatoid factor, clear factor and anti-DNA were not found. Muscle from the leg showed atrophic fibres without amyloid or plasma cell infiltrates. There was no amyloid in the lymph node biopsy. The patient has been in full time work for the last years.

Case 4

A female born in 1913 had for several years from low back pain and had a transient right leg paralysis in 1960. In early 1974 she developed sensorimotor polyneuropathy with symmetrical paresis in legs and arms. She had hypoesthesia and absent deep tendon reflexes. Ophthalmological examination showed early papilledema. Roentgenograms showed multiple dense sclerotic foci in the skeleton. These were seen on earlier films as far back as 1972. Hb was 17.0 g/100 ml, ESR 8 mm/h. No rheumatoid factor was detected. Lumbar spinal fluid showed normal pressure, protein 100 mg/100 ml and normal cell count. Immunoelectrophoresis showed monoclonal IgG lambda in low concentration in serum and spinal fluid. EMG showed a peripheral neurogenic lesion in the right leg and arm. Motor nerve conduction velocity in the right ulnar nerve was 23 m/sec. Brain scan, pneumoencephalography, cerebral angiography and myelography were normal.

Progression of the pareses and the sclerotic foci were seen in the spring of 1975 (Fig. 1). Pedal lymphography showed defects of uncertain significance in multiple lymph nodes. Hb fell to 9.2 g/100 ml and ESR rose to 54 mm/h. Spinal protein rose to 232 mg/100 ml, although the cell count was still normal. A biopsy of a sclerotic costal lesion showed plasma cell infiltration surrounded by dense infiltrations of lymphocytes adjacent to extensive sclerotic areas of bone with multiple cement lines. The knee

Table 1 Manifestations of five cases of focal sclerotic lesions

Present	absent	↑	elevated	N	normal	30 Cases collected from literature		
						No mal	Pathol	No end cause
Our cases								
1	2	3	4	5				
osteosclerosis	+	+	+	+	+			30
polyneuropathy	+	+	+	+	+			30
bone biopsy—myeloma	+	+	+	+	+			All cases histologically confirmed
lymph node biopsy—myeloma		+		(+)	(+)			
cellular film—myeloma					(+)			
bone marrow smears								
normal smears (2)	<2	~10	<5	<3	<2	0		8
Rate of diagnosis (mm/h)	3	15	15	8	41	16	14	13
Rate of diagnosis (100 ml)	15	1	15	13	15			
presence of monoclonal serum protein								10
type of monoclonal serum protein	IgG _λ	IgA _λ	IgA _λ	IgG _λ	IgA			
presence Jones proteinuria								9
monoclonal urine protein	(+)			+				3
serum protein electrophoresis	↑	↑	↑	↑	↑	0	14	7
monoclonal serum protein electrophoresis	(+)			+				8
serum protein electrophoresis (0.1)	N	N	15	N	N	17	1	1
sum of factors (Waller)				+	+			
IF anti DNA	-							
circulating immune complexes				+	+			
peripheral B-lymphocytes (2)	50			49	75			
anemias (cytopenias)	+			+	+			
osteoma or enlarged spleen (0.3)								

References 1 4 13 17 21 6 28 9 30 3 34 37 38 39 44 46 47 48 49 50 53 55 56 57 67
 cases normal or below 20. 5 cases not consistent with myeloma or below 70. Two of the cases had polyclonal
 bone biopsy one of them elevated ESR as well
 the case 0 mm/h one 15 mm/h the others below
 the bone marrow smears Hb 7.6 g/100 ml and electrolytes as well
 cases above 17 g/100 ml and one polycythemic case
 the case 7.6 and one 11.8 g/100 ml
 cases with IgA 6 with IgG and one case with Bence Jones protein in serum
 7 cases above 100 mg/100 ml one had fluid blockade
 10% case //

borderline (Table 1). Hb remained normal for a long period but anemia and elevated ESR developed in cases 2 and 4 shortly before death. Extensive marrow infiltration with low Hb and elevated ESR has been reported almost exclusively in the terminal stage (0 41 55). Thus bone marrow smears are usually normal although positive biopsies are obtained from the sclerotic skeletal foci. The marrow involvement is apparently focal rather than diffuse and may be missed by the usual bone marrow smear procedure. We therefore prefer the term osteosclerotic myeloma instead of osteosclerotic plasmocytomas.

Relationship between the features

We have collected from the literature 30 documented cases of osteosclerotic myeloma with polyneuropathy. The characteristic features of our five cases correspond well with those of these patients (Table 1) and we agree that the patients demonstrate a special form of monoclonal plasma cell neoplasia (35 57 59). On the basis of histological examination we believe that the cases are real myelomas and not results of secondary plasma cell reactions.

The characteristic features include osteosclerotic skeletal myeloma foci, a polyneuropathy re-

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ANNOUNCEMENT

International Symposium on Calcium Antagonism in Cardiovascular Therapy Experiences with Verapamil will be held in Florence Italy Oct 2-4 1980 Organized by

Secretariat AISC Assistenza Internazionale
Congresso Via G B Martini 6 Rome Italy

EORTC NCI Symposium on Nutrition of the Cancer Patient will take place in Brussels Belgium Jan 8-9 1981

The program will include invited presentations and free communications Abstracts (200-250 words) must be submitted by Nov 15 1980 and should be sent to Dr

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Bordet 1 rue Héger Bordet 1000 Bruxelles Belgium

Structure and Function of Neutrophil Leukocytes from Patients with the Immotile Cilia Syndrome

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ABSTRACT The various clinical manifestations of recently characterized immotile cilia syndrome can be traced to one cause—a structural defect of the cilia making them immotile. It was regarded of interest to examine whether other aspects of cell motility also be involved. For this reason the functions and structure of the neutrophil leukocytes were studied. Cells from eight patients with the immotile cilia syndrome and healthy controls were investigated with regard to random and stimulated motion under agarose, orientation during chemotaxis, adherence, bactericidal capacity, and chemoluminescence. Four patients showed abnormally slow migration on distances of the lead α front neutrophils. After stimulation with serum and/or an E. coli bacterial factor (BF), ascorbic acid did not reverse the defective migration. Migrating neutrophils were significantly less oriented towards the serum-staining agarose well compared with the controls ($P < 0.01$). Adherence, bactericidal capacity for *Staphylococcus aureus*, chemoluminescence, random migration, and orientation during BF-induced migration were all normal. The number of microtubules in the pericentriolar region of the neutrophil granulocytes was unusually low in four of the eight patients. We conclude that the increased frequency of respiratory tract infections in patients with this syndrome is possibly due to defects in the granulocyte motility system as well as to the defective mucociliary clearance of the airways.

Key words: chemotaxis, neutrophils, bactericidal capacity, Kartagener's syndrome, microtubules.

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Microtubules together with microfilaments are considered to be responsible for cell locomotion (1). Thus, cilia and sperm tails are capable of moving because adjacent microtubules can slide along

each other in these organelles. This movement depends on the repeated making and breaking of bonds made by a protein called dynein (2). Dynein is a high molecular weight protein and is visible in the electron microscope because it forms protrusions from the microtubules, which seem to link them together (3). The protrusions are called dynein arms and are arranged along the outer microtubules, which are also connected by the so-called spokes. If dynein arms or spokes are absent in the cilia (and the sperm tails), these are unable to move (1, 2, 11). The resulting decreased tracheobronchial clearance of dusts and microorganisms accounts for the recurrent respiratory tract infections in patient with what has been called the immotile cilia syndrome (ICS) (1, 2, 11). This may also include situs inversus and bronchiectasis and is then called Kartagener's syndrome.

The mechanisms behind granulocyte motility are not known, but microtubules extending from the centrosomes are believed to play a role (18, 30). In particular, an intact microtubular system seems to be necessary for an oriented locomotor response to cytotoxins (18). Also the adhesiveness of the leukocytes and the phagocytic capacity have been suggested to depend on microtubules (6, 8, 14). However, it is not known whether dynein arms are critical for the functions of neutrophils.

The aim of the present study was to find out

Abbreviations: BF, bacterial factor; ICS, immotile cilia syndrome; PMN, polymorphonuclear granulocyte.

The results were presented in part at the Annual Meeting of the Swedish Medical Association, Dec. 1978 and at the Int. Congr. of Haematology, Hamburg, West Germany, 1979.

Table 1 Clinical data on eight patients with the immotile cilia syndrome

Case numbers are the same as in ref 11 and 19 except for patient 15 not described before. Cases 1 and 2 are brothers. Sperm tails from case 4 were normally equipped with dynein arms but lacked probably radial spokes. Dynein arms were present in sperm tails from case 14 but probably in reduced numbers.

Case no	Sex	Age (y)	Recurrent infections	Fertility	Situs in versus
1	♂	40	+	+	+
2	♂	74	+	+	-
3	♂	35	+	+	+
4	♂	35	+	+	-
7	♀	30	+	?	+
13	♀	29	+	?	+
14	♂	35	+	+	+
15	♀	29	+	?	+

whether orientation during neutrophil locomotion stimulated and random migration, bactericidal capacity, chemoluminescence and adherence are abnormal in patients with documented ICS including ultrastructural defects of the dynein arms or radial spokes. We have also attempted to relate the migratory responses of the neutrophils to the number of centriole associated microtubules determined by electron microscopy.

STUDY POPULATION AND METHODS

Patients. Eight patients (5 men, 3 women) with documented ICS were investigated with respect to their neutrophil function. Further details of the patients described in previous publications (11, 19) are given in Table 1. All patients exhibited no or low tracheobronchial clearance, six lacked dynein arms, one had reduced numbers in cilia and/or sperm, and one lacked axonemal spokes. All had a history of recurrent infections of the upper and lower airways with chronic expectoration of mucopurulent sputum. Patient 3 has a massive history of allergy previously associated with asthma and now with generalized

eczema. He has been treated with steroids but not

All patients with recurrent infections were frequently treated with antibiotics, mucolytics and theophylline derivatives. All drugs were however withdrawn for 2 days prior to blood sampling. None had recent infection as evidenced by the case history and ESR (2-16 mm/h in all except patient 3). All had normal Hb concentrations, leukocyte counts, serum electrolytes and renal function tests and urinalyses. Pa had slightly elevated γ -ASAT and S-ALAT levels, albuminuria. His other laboratory tests were normal.

The study included paired healthy age and sex matched controls. In addition to these controls, 11 blood bank donors were used for comparison.

Leukocyte preparation. Erythrocytes in heparinized blood (10 IU heparin (without preservative) per ml) were sedimented in 4.5% dextran T 500 and the leukocyte supernatant was centrifuged at 500 g for 6 min. The pellet was washed twice in heparinized 0.9% saline (10 IU heparin per ml) and the leukocytes were suspended either in Hank's balanced salt solution containing 1% gelatin for the bactericidal and chemoluminescence assays or for the chemotaxis assays in a 31% buffer (Sigma St. Louis, Mo, USA) dissolved in 199 medium (State Bacteriological Laboratory, Stockholm) to make appropriate neutrophil concentrations.

Neutrophil assays. The bactericidal capacity was analysed as described previously (22). In short, neutrophils were mixed with suspensions of *Staphylococcus aureus* serum. The mean concentrations in the test tubes were 2.5×10^6 (range $2.2-2.8$) of bacteria per 10% only forming units/ml and of serum 10%. After 45 min incubation at 37°C, samples were removed for estimation of viable bacteria. The results are given as percentages of living bacteria after 45 and 90 min incubation of the initial counts. The reference values established on the basis of analyses of 103 healthy blood donors are given in Table II. The reproducibility of the method has been presented elsewhere (23).

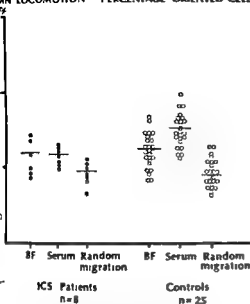
The granulocytic oxidative metabolism was quantified with the luminol augmented chemoluminescence as described previously (12). Granulocytes (2.5×10^6 (10%) living *Staphylococcus aureus* (70×10^6) and 10^{-6} M) were incubated for 20 min at 37°C. Maximal chemoluminescence was then measured with a photomultiplier and the results are expressed in mV.

Adherence was assayed by the nylon fiber coil method described by MacGregor et al (17) and Palm et al (24). The results are given as the percentage of neutrophils adhering to the fibers. The reference values

Table II Neutrophil functions in patients with the immotile cilia syndrome and in controls (mean \pm S.D.)

	Patients	Controls	Reference values (mean \pm 2 S.D.)
Bactericidal capacity (% colony forming units)			
After 45 min	53 \pm 19	49 \pm 18	27-148
After 90 min	23 \pm 12	22 \pm 8	0-5-34
Chemoluminescence (mV)	188 \pm 48	207 \pm 52	Not established
Adherence (%)	53 \pm 18	41 \pm 22	22-95

RANDOM LOCOMOTION—PERCENTAGE ORIENTED CELLS



1 Percentage migrating granulocytes showing orientation. The mean variation of the determination is 5%. A statistically significant difference was noted between patients and controls when migration was induced by serum (0.01). In the extended control series orientation during migration towards serum is significantly higher (0.01) than towards the bacterial factor (BF) and during spontaneous migration ($p < 0.001$).

PMN SPONTANEOUS MIGRATION AND LOCOMOTION INDUCED BY BF



2 Stimulated and spontaneous locomotion of neutrophils from patients with the immunotile-cilia syndrome, controls and extended control series measured using the leading front technique (a) Spontaneous and BF induced locomotion --- = Reference values (± 2 S.D.)

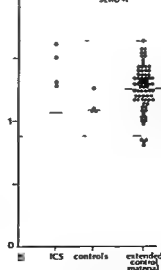
given in Table II. The mean day-to-day variation (S.E. value) for a single subject is 4%.

The random migration and stimulated locomotion induced by two cytotoxins were assayed with a modification of a method measuring neutrophil migration under agarose (20, 28). Briefly, an agarose solution with a final albumin concentration of 0.08% was allowed to gel in Petri dishes. Six series, each consisting of 3 wells, were punched in the agarose. The diameter of one well was 3 mm and the distance between the centers of two adjacent wells 5 mm. A 10 μ l sample of a leukocyte suspension containing approximately 10×10^6 neutrophils/l (range 8.5–12.7) was filled in the central well. Chemotactic factors, either a sterile *E. coli* bacterial filtrate (BF) (24) or pooled human AB serum (22) were filled in the outer well. Tissue culture medium was added to the inner well.

The dishes were incubated for 3 hours at 37°C in an atmosphere of 5% CO_2 . After fixation with methanol and staining with hematoxylin and fuchsin, the agarose dishes were removed and the distance migrated by the leading front neutrophils was measured by microscopy and given in mm. In addition, we counted the number of cells migrating towards the chemotactic factors and towards the tissue culture medium using an ocular containing a grid of vertical and horizontal lines (21). The number of cells within each grid space was counted and the location of the maximal cell density, i.e. the grid space containing the highest number of cells, was determined.

The orientation of lamellipodia and nuclei of 600 neutrophils migrating towards the cytotoxin and tissue culture medium wells was estimated by microscopy for each subject. The cells were considered polarized in the gradient when

PMN LOCOMOTION INDUCED BY SERUM



for the extended control series, for BF induced locomotion they are 0.65–1.34 mm and for spontaneous locomotion 0.09–0.28 mm (b) Serum induced locomotion. The reference value for the latter (—) is 0.88–1.63 mm.

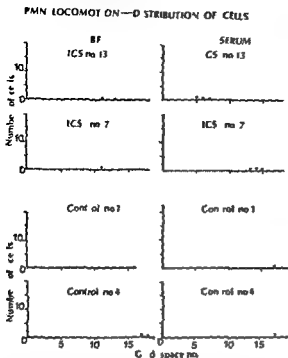


Fig. 3. Number of cells within each grid space in two patients and two controls calculated as described by Orr and Ward (1971). The border of grid space 1 touches the neutrophil well and the space with the highest number is closest to the cytotoxic well.

the nuclei were in the rear and the anterior lamellipodia located within a 90° sector open towards the cytotoxic well. The orientation of migrating neutrophils from 23 healthy donors including the matched controls are shown in Fig. 1.

No difference in the distance to the leading front or the location of maximal cell density was found when neutrophil suspensions on concentrations varied between 7 and $1.6 \times 10^6/l$. The day-to-day variation (S.D. in per cent of mean) of granulocyte migration for three subjects assayed as above was 6%. The reference values for neutrophil migration measured as the leading front were established from the analyses of apparently healthy blood bank donors and members of the laboratory staff and are given in Fig. 2.

Neutrophils from patients and controls were also incubated with ascorbic acid (10 mmol/l) (Merck, Darmstadt, Germany) at 37°C for 15 min prior to application on the agarose dishes with BF as chemotactant. Stimulated and random migration assessed as above with the leading front distance were compared with cells treated in a similar way except for ascorbic acid.

Electron microscopical methods. Leukocyte preparations from patients and controls were processed with identical techniques at the same occasions. Primary fixative was 4% glutaraldehyde in 0.05 M cacodylate buffer to which 0.015% calcium chloride and 4% sucrose had been added. After fixation the samples were rinsed in the buffer, postfixed in a 1% osmium tetroxide solution in the same buffer, dehydrated in a graded ethanol series and

embedded in Epon 812. Sections were cut as a ultrathin section (600 Å) and section stained in a standard fashion with uranyl acetate and lead citrate.

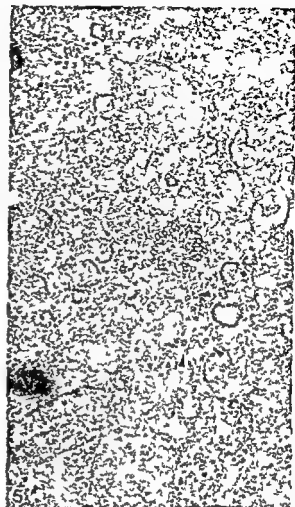
The number of microtubules was recorded as follows. Granulocytes which had been sectioned at the level of one or both centrioles were recorded in the electron microscope at a magnification of $\times 15000$. This magnification was too low to permit the microscopist to select microtubules yet high enough to permit a count of the number in the (three times) enlarged photomicrographs. The area of cytoplasm around the centrioles recorded as 15 μ^2 . Coded specimens were selected in the electron microscope to further permit a non-biased sampling.

RESULTS

Neutrophils from the patients showed a significant decrease compared with the controls in their orientation towards the serum- or cytotoxic well ($p < 0.01$, Mann-Whitney U-test). Orientation towards the BF-containing wells was similar in patients and controls ($p > 0.05$). As there was no statistically significant difference ($p > 0.05$, Mann-Whitney test) between the paired controls and the extended controls with regard to locomotion distances, adhesion, chemotaxis and bactericidal capacity (Table II), several patients showed values outside reference values for neutrophil locomotion measured as the distance to the leading front. Neutrophils from patients 7 and 13 were below reference values for migration induced by BF (Table 1a); from patients 13 and 14 they were below reference values for the lower limit of the reference values for locomotion induced by serum (Table 1b). On the other hand, patient 1 showed a high random mobility and BF-induced migration (Fig. 1a). Four of the eight patients exhibited abnormal cell locomotion after stimulation with BF and serum. While one patient demonstrated locomotion induced by both cytotoxins, the other three had low values for either BF or serum.

After stimulation with ascorbic acid the distance to the leading front was not more enhanced in the ICS patients than in the controls.

The distribution of cells along the microtubules was determined for patients and controls. The values for two patients and two controls are shown in Fig. 3. The location of maximal cell density was assessed from determinations of this kind and yielded a lower migration for patients 7 and 13 than for any control subject's neutrophils when



4 and 5 Transversely sectioned centrioles in neutrophils from patient 2 (Fig. 4) and a healthy control (Fig. 5). In some cells some microtubules run parallel with the centriole and hence appear cross-cut (arrow heads) in the electron micrograph. In other cells they run perpendicular to the centriole and are seen

in longitudinal views (arrows). No systematic difference was found in orientation of microtubules or appearance of centrioles between patients and controls. Magnifications $\times 63\,000$ (Fig. 4) $\times 67\,500$ (Fig. 5).

was induced by BF and similarly for patient 13. Chemotactic migration was induced by serum. The neutrophils had essentially the same ultrastructure as described by Malach et al. (18) and will not be presented in detail. Our findings differed somewhat from those of Malach et al. (a) The system of filaments with a diameter of about 100 nm (called thick filaments by Malach et al. and intermediate filaments or intermediate sized filaments by most others) formed bundles rather than isolated strands in cells in which they occurred. Such bundles were seen in neutrophils both from controls and ICS patients. The significance of these filaments and of the dif-

ferences in their arrangements is not understood and will not be discussed further. (b) The number of microtubules per unit area around the centrioles was lower than in their study. A possible explanation is that we examined the ultrastructure of unstimulated neutrophils whereas Malach et al. studied stimulated ones. The specimen preparation technique is also likely to influence the number and appearance of the microtubules.

The centrioles and the microtubules around them were studied in further detail. Like in other types of cells the centrioles consisted of nine microtubular triplets surrounded by an amorphous substance (Figs 4-5). There were lateral extensions from one



Figs 6 and 7 Pairs of centrioles in neutrophils from two controls. In one cell the two centrioles have parallel axes (Fig. 6) in the other they are at right angle to each other (Fig. 7). There was no systematic difference between patients and controls with respect to orientation of centrioles

relative to each other or relative to the closest nuclear lobe. The longest centriole in Fig. 6 has lateral positions. centriolar satellites (arrows). IF = some intermediate filaments. Magnification $\times 49,500$.

of the centrioles, so called centriolar satellites (Fig. 6).

Special attention was paid to the orientation of the centrioles. In the control group as well as in the patient group the two centrioles could lie more or less in line (Fig. 6) or be perpendicular to each other (Fig. 7). Nor was the orientation of the centrioles relative to the nearest nuclear lobe found to be constant.

The centrioles were surrounded by a finely granular region from which ribosomes and other organelles were excluded. Some microtubules could be seen in this region, although most of them seemed to originate from the distal portions of the satellite

and radiate outwards. The pericentriolar microtubules recorded as specified in Method were counted and the absolute numbers and percentages are given in Table III.

Another way of expressing the number of microtubules is to give the median number of microtubules in the examined neutrophils from each subject. They were 0.5, 1, 1, 1.5, 2.5 and 7 for controls and 0, 0, 0, 0, 1, 1, 2 and 3 for the patients. Patient 14 (with a low motility score) had the highest value, 3, and patients 3 and 7 (also with low motility scores) had 0. It is to be noted that the four lowest median values were found in patient group. It can be calculated that there is

Table III Number of microtubules (MT) in the pericentriolar region

Neutrophils with												
0 MT		1 MT		2 MT		3 MT		4 MT		>4 MT		Total (n)
n	%	n	%	n	%	n	%	n	%	n	%	
9	60	2	13	1	7	0	0	11	0	3	20	21
14	67	11	0	2	9	1	5	2	9	2	9	21
11	85	11	11	1	8	11	0	0	0	1	8	13
5	24	4	19	4	19	2	9	3	14	3	14	21
15	60	2	8	2	8	0	0	4	16	2	8	25
5	38	3	23	1	8	1	8	2	15	1	8	13
1	7	2	13	2	13	3	20	1	7	6	40	15
4	40	3	30	1	10	1	10	0	0	1	10	10
Controls												
2	13	3	19	3	19	1	6	1	6	6	38	16
0	0	1	10	4	40	2	20	2	20	1	10	11
7	44	3	19	2	13	2	13	1	6	1	6	16
4	40	1	10	1	10	2	20	2	20	0	0	10
7	44	5	31	1	6	2	13	0	0	1	6	16
5	50	3	30	1	10	0	0	1	10	0	0	10

probability that chance alone is the reason for our lowest median values being found in the patient group. The difference between the two groups is statistically significant although large enough to be worthy of a further investigation. Although not of interest for the main topic of this paper, it may be mentioned that so called parallel tubular arrays were found in the lymphocytes from patients 13 and 14 (Fig. 8) but not in lymphocytes from the controls. This is mentioned here because it has been suggested that these parallel tubular arrays are characteristic of lymphocytes from persons who have been treated with steroids (25, 26). At least patients 2 and 14 deny having been on such therapy.

DISCUSSION

This syndrome is characterized by immotile cilia in the airways and immotile spermatozoa in males and often situs inversus (Kartagener's syndrome) leading to decreased resistance to infectious agents entering the airways and also to male infertility. Some ultrastructural abnormalities have been suggested to be the cause of the immobility such as absence of dynein arms or spokes between the microtubules (11, 31). The patients studied here had such abnormalities documented previously (1, 11). The present study has shown that additional defects may also be present in some ICS patients. A

significantly lower mean rate of orientation in the serum gradient, a low number of centrioles associated microtubules in the resting granulocytes in some of these patients and an abnormally short distance migrated by neutrophils from 4 out of 8 tested patients point at a more widespread defect of the cytoskeleton than previously anticipated. The defective neutrophil orientation and migration might also help to explain the increased susceptibility to infectious agents in the airways eventually leading to bronchiectases.

Previous studies on patients with ICS and/or Kartagener's syndrome have focused mainly on the ultrastructure and function of cilia and spermatozoa (1, 2, 3, 11, 27, 31). However, some studies of neutrophil functions have been reported. Lupin and Misko (16) noted that the bactericidal capacity was slightly reduced in their two patients with ultrastructurally abnormal cilia. Caleb et al. (9) reported a case with situs inversus, repeated infections and defective neutrophil chemotaxis. They suggested that defects of the centriole or dynein arms could possibly account for their findings although they did not report any studies of the ultrastructure or function of cilia and neutrophils. Hence, as the present study demonstrated migratory defects were found in neutrophils from half of the patients with a well defined ciliary immotility whereas no defects of the bactericidal mechanisms were noted. The reason for

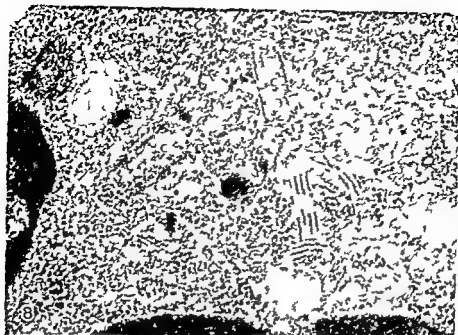


Fig. 8 Two centrioles and some parallel microtubules in a lymphocyte from patient 14. Such an abnormality is observed in some persons who have received steroid treatment. This patient has never received such treatment. Magnification $\times 48,000$.

this discrepancy from the other Kartagener patients described by Lupin and Misko is not known.

The role of microtubules for leukocyte functions have been documented in several ways. In e.g. Chediak-Higashi's disease—a suggested defective assembly of microtubules in leukocytes results in low chemotaxis, adherence, degranulation of lysosomal enzymes and a retarded bactericidal ability (6, 7). This impairment of migration may in some patients be reverted with drugs such as ascorbic acid increasing, e.g. the intracellular levels of cGMP (6, 7, 13). In contrast to what has been found in Chediak-Higashi's disease, our patients did not show any defects in other functions than stimulated migration. Furthermore, neutrophils showing abnormally short distances migrated by the leading front cells were not enhanced more by ascorbic acid than the normally migrating neutrophils from patients and controls. It could hence be argued that these defects do not seem to be due to mechanisms responsible for the defective assembly of microtubules as in patients with Chediak-Higashi's disease, where orientation of migrating neutrophils seems not to have been measured.

In contrast to cases with small numbers of microtubules in the neutrophils and poor migratory capacity, Gallin et al. (14) described a 7-year-old patient with markedly increased number of centriole-associated microtubules in the migrating neutrophils and a defective cell locomotion to-

gether with delayed bactericidal activity and low adherence. Thus abnormally low and various neutrophil functions may be associated with too few as well as too many microtubules.

The assay employed here for measurement of neutrophil locomotion is principally similar to that described by Nelson et al. (20), Repo (28) and Ward (21). We have also analyzed the orientation of the neutrophils in the cytotax assay under the agarose—an additional information is easily obtainable in the same assay as the chemotaxis variables (33). It is interesting that the orientation of migrating neutrophils was significantly lower in patients with ICS than in controls. Centriole-associated microtubules have been suggested to be important for cell orientation during locomotion (4). Agents affecting microtubules, such as vinblastine and colchicine, have been shown to decrease cell orientation (18, Palmblad et al. unpublished observations). Whether this abnormally low orientation seen in all our patients is related to the number of microtubules, some defects in the orientation of centrioles or some other structural defect is not known.

For the stimulation of neutrophil locomotion we have used two different cytotaxis methods with different results in some patients. The nature of these differences is not known; they may be related to neutrophil surface receptors. Since the neutrophil surface is equipped with receptors for C^3 and

ated compounds generated in serum in contact with agarose) and synthetic tripeptides (5) (similar to BF) it is conceivable that the neutrophils could migrate differently after stimulation of these receptors due to its actual sensitivity. It has recently been demonstrated that defective neutrophil locomotion might be found after stimulation with one cytotoxic whereas it is normal after activation with another (3). These findings emphasize the importance of studying neutrophils with several different motile factors. Also methods of distinguishing cause of a defective locomotory response are needed to find out whether there is a primary deficiency of the locomotor structures or whether the defects are secondary to other conditions leading to activation of the cells. We could not find any studies suggesting that recent infections would have an influence on the cause of impaired neutrophil migration in patients. Only little is however known about errors in the systems of cell locomotion. The analyses of the degree of orientation of cells in a gradient is an assay which might be employed shed light on mechanisms which are suggested depend on microtubules.

ACKNOWLEDGEMENTS

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Effect of Long-Term Treatment with Human Leukocyte Interferon on Various Laboratory Parameters

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TRACT Leukocyte interferon was given by intramuscular injection as adjuvant therapy to 9 patients with osteosarcoma. The dose was 3×10^6 standard units daily for the first month, and then 3 times a week for the next 17 months. Blood samples were drawn at intervals. A number of routine tests during the 18-month course of interferon administration and during the subsequent 18 months. On withdrawal of the interferon treatment the mean Hb concentration rose significantly and the mean ESR fell significantly. There was no significant change in the leukocyte and platelet counts or in the alkaline phosphatase, alanine aspartate aminotransferase or plasma protein levels.

Keywords: interferon, blood chemistry
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Human leukocyte interferon has been used during the 70s at the Karolinska Hospital for treatment of a number of tumour diseases (13, 14). In Dec 1971 a clinical trial has been conducted with 14 patients with osteosarcoma have received interferon as an adjuvant form of treatment (13). After a period of 6 years 9 patients had completed an 18-month course of interferon therapy and were followed for a further 18 months. To ascertain the effect of long-term interferon therapy clinical examinations (6) of these patients were supplemented with regular laboratory tests. At the beginning of this therapy all the patients were suffering from an untreated tumour disease. As the laboratory values under examination might be influenced by the tumour disease and the treatment of it—especially surgery and blood transfusions—the study was confined to periods during and after interferon

course when the effect of such factors appeared to be least marked.

STUDY POPULATION AND METHODS

Patient series The 9 patients studied had osteosarcoma for which they had been receiving interferon as an adjuvant to conventional tumour treatment. They had no history of disease prior to the development of osteosarcoma. At the beginning of the course of treatment the patients (6 males and 3 females) ranged in age from 10 to 28 years. The intended primary treatment of the tumour was radical operation preceded in 6 cases by radiotherapy. The times for these measures and the time or replacement of concentrated interferon given initially by more purified preparations are given in Fig 1. The preoperative radiotherapy was given as cobalt-60 irradiation. The treatment schedule was 2 Gy daily (tumour dose) 5 days a week for 4 weeks by which time a total tumour dose of 40 Gy had been administered. After an interval of 2 weeks 3 of these patients were given a further 24–28 Gy (tumour dose) following the same fractionation schedule.

Interferon preparations The interferon was produced by *in vitro* stimulation of blood bank leukocytes with Sendai virus (2, 11). The course was started immediately after the diagnosis had been verified and thus before any other treatment for the tumour disease had been given. The interferon was administered as an intramuscular injection of 3×10^6 standard units daily for the first month and thereafter 3 times a week for a further 17 months. This dose of interferon yields levels of 20–30 U/ml plasma (3). The treatment was started with concentrated interferon (2, 11) and if troublesome side-effects occurred (6) it was replaced by a more purified preparation (2, 11). The concentrated interferon contains $1-2 \times 10^6$ standard U/ml and its specific activity is $1-5 \times 10^6$ standard U/mg protein. Purified interferon contains $5-20 \times 10^6$ standard U/ml and $1-5 \times 10^6$ standard U/mg protein.

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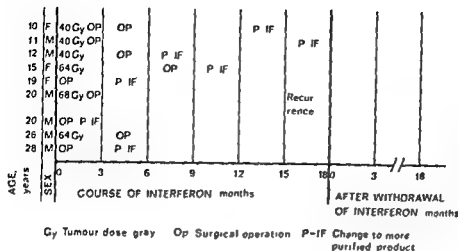


Fig 1 Time of in therapy in relation forms of treatment change to more purified product

All 9 patients underwent regular clinical examinations and comprehensive laboratory tests. During the interferon therapy they were checked 12-17 times. During the following 18 months each patient was examined 5-12 times.

Two patients developed local recurrence: one of them in the last month of the interferon therapy and the other 2 years after the course had been completed. As the test results for these 2 patients did not differ noticeably from those for the other patients, they were not excluded from the series.

Laboratory tests. All analyses were performed at the routine laboratories of Karolinska Hospital. The test methods used and the reference values of the blood variables are given in Table 1.

Statistical analysis. The 18-month course of interferon treatment and the subsequent period of the same length were divided into 3 month periods for each of which the mean values of the respective variables were calculated for the individual patients. Every effort was made to compare steady state. Thus—in order to avoid the influence of the tumour burden, radiotherapy, operation and any blood transfusion etc. during the initial course of the disease—the statistical analysis was confined to the last 9 months of

the interferon therapy. To avoid any unreliability of the interferon therapy, the first 3 months after were disregarded. The mean values for the three periods of interferon therapy were compared with the respective values for the three post-month periods. A difference was regarded as significant ($p < 0.01$). The aggregate results of the laboratory formed over the 3 year period are presented in Fig 2a, b and c.

RESULTS

A significant difference between the values recorded during and after the interferon therapy was found only for ESR and Hb concentration.

Erythrocyte sedimentation rate. The values during the last 9 months of the interferon therapy were significantly higher than those for the post-month periods after withdrawal of interferon therapy. Over the whole period of interferon treatment there was a considerable drop in the ESR after

Table 1 Laboratory methods

Parameter	Method	Reference value (ad)
ESR (mm/h)	Routine method (modified from Westergren)	Men 1-15 women 1
Hb (g/l)	Routine method cyanmethaemoglobin	Men 140-170 women 150
Leukocytes ($\times 10^9/l$)	Routine method manual	4.0-9.0
Platelets ($\times 10^9/l$)	Routine method manual	150-400
Electrophoresis	Agarose gel (Laurell ref 8)	
Albumin (g/l)	Immunological technique (turbidometry)	37-52
Haptoglobin (g/l)	Immunological technique (turbidometry)	0.4-2.5
IgG (g/l)	Immunological technique (turbidometry)	7.0-15.0
IgA (g/l)	Immunological technique electroimmunoassay	0.8-3.8
IgM (g/l)	Immunological technique electroimmunoassay	0.4-2.0
ASAT ($\mu\text{kat/l}$)	According to Scand Enzyme Comm (ref 12)	<0.70
ALAT ($\mu\text{kat/l}$)	According to Scand Enzyme Comm (ref 12)	<0.70
ALP ($\mu\text{kat/l}$)	According to Scand Enzyme Comm (ref 12)	0.8-1.0

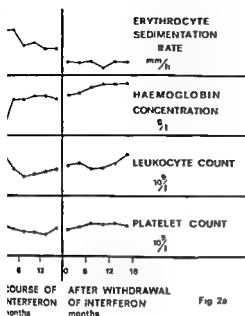


Fig 2a

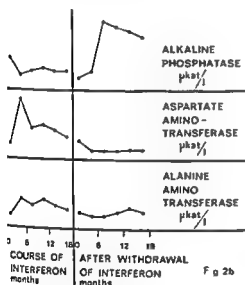


Fig 2b

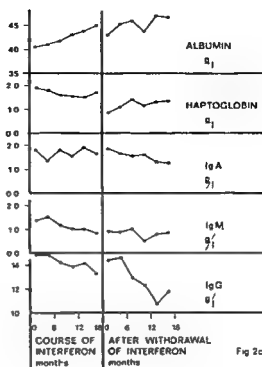


Fig 2c

Fig 2 Course of the blood variables during and after interferon therapy. O = mean of the single determination made in each of the 9 patients before interferon therapy ● = mean of the 9 means (each of several determinations in one patient) for the 3 month period

of interferon there was a further decrease by a fairly steady state (Fig 2a) haemoglobin concentration. The Hb level rose to a plateau at the beginning of the interferon treatment and remained fairly constant throughout the course (Fig 2a). On withdrawal of interferon there was a further increase to a new level. The difference between the mean values for periods during and after treatment was statistically significant ($p < 0.01$). Leukocyte count. There was no significant difference between the counts for the 9 month

periods. Mean counts of about $5-6 \times 10^9$ cells/l were recorded for the 18 months of interferon therapy and the subsequent 18 months (Fig 2a).

Platelet count remained practically constant at $200-300 \times 10^9$ platelets/l throughout the 3 year period (Fig 2a).

Alkaline phosphatase. In 3 of the patients the difference between the values during and after the therapy was small. In the other 3 patients, aged 10, 11 and 12 years, a high level was recorded during the interferon treatment and a steep increase after withdrawal of the drug. The high levels recorded in

these 3 patients rule out a meaningful statistical analysis (Fig 2b)

Alanine and aspartate aminotransferase There was no significant change in the levels of these enzymes on discontinuing the interferon therapy (Fig 2b)

Plasma proteins The analysis of the plasma proteins by electrophoresis disclosed no evidence of a significant change when discontinuing the interferon therapy (Fig 2c)

DISCUSSION

Interferon has been increasingly applied in clinical treatment. Administered in i.m. injections it has been used in cases of virus infections and malignant diseases. The ESR has been reported to be higher during interferon therapy (4-14). Bone marrow suppression with low granulocyte, platelet and reticulocyte counts has been noted in some studies (5-9, 10) but not in others (1-4, 7). It has been suggested that the suppression is dose-dependent (5-9). An elevated transaminase activity during interferon therapy has been reported in patients with hepatitis malignant disease involving the liver and congenital virus infections (1-5, 7). The interval between injections, the dose and the duration of the therapy in these studies have varied widely. The treatment has often been introduced in an aggressive stage of the disease. In addition, some of the patients had recently undergone radiotherapy for underlying malignant disease and it is therefore difficult to judge whether any trends disclosed by the laboratory tests should be ascribed to the interferon therapy alone.

Despite the small number of subjects in this study, the long follow-up time with relatively frequent sampling enables trends in the material to be analysed in relation to the interferon therapy. The study was largely confined to periods during the treatment when influence of other factors would seem to be least marked. As each patient served as his own control, the skew age and sex distribution may be disregarded and no control group is required.

The implications of the observed significant difference in ESR over the relevant intervals are unclear since there was a steady decrease in ESR from the time of initiation of the treatment. We thus cannot be sure that posttherapy reduction was solely due to the withdrawal of the interferon (Fig 2a). Whether this trend is ascribed to a change over

to the more purified interferon III also to say since this was made at different times in the course of the treatment (Fig 1).

During the first 3 months of the course of interferon therapy the Hb concentration rose to a plateau. After withdrawal of the drug there was a fall to a new plateau. The differences between mean values for the compared 3-month periods before and after the therapy were statistically significant.

As regards the alkaline phosphatase, the mean for the III patients increased after interferon therapy had been discontinued (Fig 1). On the other hand, the individual difference periods in question were not statistically significant. The high alkaline phosphatase level both before and after interferon therapy is accounted for by the fact that 10 of the patients aged 10-11 and 12 years. In the last 9 months of the treatment the mean for these patients was 8.4 against 13.5 $\mu\text{kat/l}$ in the first 9 months after withdrawal of the drug.

There was no significant difference between mean values of ASAT and ALAT before and after withdrawal of the drug. Nor was there a significant change in the leukocyte and platelet counts throughout the follow-up period.

In spite of the significantly elevated IgG during the interferon treatment, there was no statistically significant change in the level of plasma proteins. Throughout the follow-up period the IgG was high in relation to age, while the IgA was on the low side. The IgG fell during the course of treatment but when the drug was withdrawn there was a transient increase.

Taken together, long-term treatment of sarcoma patients with human leukocyte interferon in the dose used did not affect marked biochemical variables examined. Likewise, severe symptomatic side effects were encountered (6). In studies on patients with other types of cancer receiving higher doses of interferon, the therapy was found to exert an effect on blood components. There is a clear need for further investigation of the effect of interferon therapy in malignant diseases and to examine the early phase of treatment free from interference from other factors.

ACKNOWLEDGEMENTS

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Immunological Studies on Human Thymus

Occurrence of Distribution of Immunoglobulin and Complement Receptors
in Myasthenia Gravis and Control Patients

Birger Christensson Georg Matell and Peter Hjerfald

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TRACT Suspended cells and tissue sections of myasthenia gravis (MG) and control thymuses characterized for surface expression and histological distribution of immunoglobulin (Ig) and receptors for sheep erythrocytes (SE) the Fc part of (Fcγ) and of IgM (Fcμ) and complement factors and C3d (CR). In sections the cortical areas of both MG and control thymuses expressed SE as Fcγ receptors whereas medullary areas only showed weak SE binding. In contrast to control patients MG thymuses also contained Ig+ cells confined to nodular areas of the thymic medulla. These were stained for both IgG and IgM and were identical with respect to light chains. In several IgD was found as well. In addition the nodular areas were positive for C3b and C3d receptors. In sections these Ig+ CR+ nodules corresponded morphologically characteristic lymphoid follicles. B cell follicles were observed in variable number in all MG thymuses whether hyperplastic or normal histology. Five thymomas gave homogeneous results with regard to immunological markers. Comparison of methods showed that receptor and Ig induction on sections was more sensitive than corresponding tests on thymus cells in suspension.

Word human thymus myasthenia gravis immunological markers immunoglobulin complement receptors

Med Scand 08 161 1980

About 75% of patients with myasthenia gravis have hyperplastic thymuses with follicular structures and about 10% have thymomas (7). Histological observations and immunofluorescence studies of cells recovered from myasthenic thymuses (1, 2, 9, 11, 15) have suggested an abnormal B cell proliferation in MG thymuses. There is also evidence for both humoral (16) and cell

mediated (10, 17, 19) autoimmune reactivity in MG. However, the relevance of the thymic changes in the pathogenesis of MG is far from clear although suggested by the beneficial effect of thymectomy (18).

In the present study the correlation between histological changes and the presence and tissue distribution of T and B cell markers was investigated and compared with that of thymuses from control subjects.

MATERIAL AND METHODS

Thymus biopsies from 7 thymectomized MG patients and from 13 patients undergoing open cardiac surgery (controls) were studied. The biopsies were divided and pieces for immunological studies were quickly frozen in streamer CO₂. The frozen tissues were kept at -70°C until they were cryosectioned in 5–10 μm thick sections. The sections were transferred to coverslips and allowed to dry in room temperature for one hour. In some experiments thymus specimens were also resuspended and viable cells were tested. Morphology was examined in conventional histological preparations.

Abbreviations: MG myasthenia gravis SE sheep erythrocytes Ig immunoglobulin AET amnoethylthionium bromide hydrobromide PBS phosphate buffered saline IFL immunofluorescence PAP = peroxidase diaminobenzidine tetrahydrochloride OEA ox erythrocytes coated with antibody of the IgG class OEA-M ox erythrocytes coated with antibody of the IgM class OEA-MC ox erythrocytes coated with antibody of the IgM class and complement factor C3b complement factor C3b C3d complement factor C3d Cr complement receptor for Fcγ receptor for the Fc part of the IgG molecule Fcμ receptor for the Fc part of the IgM molecule FITC fluorescein isothiocyanate F(ab)'₂ an Ig molecule lacking the Fc fragment C5 complement factor C5 C6 complement factor C6 FCS fetal calf serum OE ox erythrocyte ALP alkaline phosphatase ACP acid phosphatase NSE non specific esterase



Fig. 1 Frozen section of control thymus showing dense binding of AET treated sheep erythrocytes over cortical areas but only sparse binding over medullary areas

Indicator systems

Receptors for sheep erythrocytes (SE) were demonstrated with SE treated with AET (2-amino-ethylthiuronium bromide hydrobromide, Sigma) according to Kaplan and Clark (14). In short, one volume of packed SE were incubated with four volumes of 0.143 M AET dissolved in deionised water at pH 9 for 15 min at 37°C, washed five times in phosphate buffered saline (PBS) and resuspended to 1% in PBS with 0.1% gelatin.

For demonstration of Fcγ and Fcμ receptors on erythrocytes (OE) were incubated with subagglutinating doses of anti-OE IgG and IgM, respectively, for 30 min at 37°C. The resulting OEAG and OEAM indicator cells were washed three times in PBS and resuspended to 0.5% in PBS gelatin as previously described in detail (8).

For demonstration of C3d receptors OEAM were coated with C3d by incubation with a C3 deficient mouse serum diluted 1:2 at 37°C for 15 min. For demonstration of C3b receptors C6 deficient rabbit serum was used. The EAMC indicator cells were subsequently washed three times and resuspended to 0.5% in PBS gelatin. The gelatin containing buffer was shown to diminish unspecific binding (8).

Receptor indication

Suspended cells were incubated with respect to indicator erythrocyte system according to conventional techniques (17). For concomitant double indication of SE and Fcγ receptors either indicator system was labelled with fluorescein isothiocyanate (FITC).

The frozen tissue sections were incubated according to the closed chamber technique of Tønder et al. (26). In short, the concavity of a microculture slide was filled with indicator cells. A coverslip was adapted over the cavity with the section on the inside and the glass slides were then pressed together making a closed chamber containing both the section and the indicator erythrocytes. Incubation with the coverslip down for 30 min allowed the indicator cells to sediment on and bind to the receptors. After incubation the chamber was turned coverslip up and erythrocytes not adhering to the section were allowed to

settle for 15 min. Subsequently the sections were examined under microscope and the density and percentage distribution of the adsorbed indicator erythrocytes determined. The density was evaluated from the distribution pattern denoted as medullary and as nodular or diffuse. For photography sections were fixed in 3% glutaraldehyde and stained with benzidine and toluidine blue for easy differentiation of erythrocytes and thymus tissue.

Immunofluorescent (IFL) staining

Unfixed cryosections were incubated in PBS with 5% fetal calf serum (FCS) prior to incubation with sera. Appropriate dilutions of antiserum against the classes and light chains were used. FITC-conjugated (Fab) fragments of anti-IgM, anti-IgD, anti-IgG and anti-λ were purchased from Hællstedt Labo. Anti-IgG FITC (Fab) fragments were obtained from the National Bacteriological Laboratory, Stockholm. Sections were incubated with antibody at room temperature for one hour and washed in PBS with FCS before mounting in buffered glycerol. They were examined in a Zeiss microscope with epillum at ×100 and ×400 magnification.

Immunoperoxidase (PAP) staining

Paraffinized sections were consecutively treated with rabbit antibodies against human Ig classes, goat anti-serum and preformed rabbit anti-peroxidase (PAP) complexes according to the technique of Sjöström (24). Bound peroxidase was demonstrated by treatment with diaminobenzidine.

Histochemistry

Conventional methods were used for staining of cryosections for demonstration of alkaline phosphatase (ALP), phosphatase (ACP) (5), nonspecific esterase (NBE) and peroxidase (27).

RESULTS

SE, C3 and Fc receptors

Eleven thymus biopsies from patients under cardiac surgery (control thymuses) were investigated for SE, Fcγ, Fcμ, C3d and C3b receptors. In all cases receptors for SE could be demonstrated. In frozen thymus sections using untreated SE, binding was stronger and the adsorption denser when AET-treated SE were used. The adsorption was dense in cortical areas and only sparse in medullary regions (Fig. 1). Cortical areas also revealed a dense binding of OEAG but virtually no binding was found in the medulla. Untreated AET-treated OE on the other hand did not bind to the thymus sections. The cortical binding of AET and OEAG was on serial sections seen to cover the same areas. All control thymuses

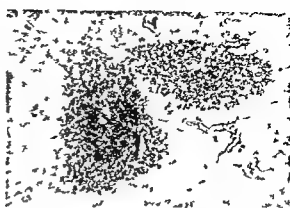
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	7	q	+++c	+++c	
	9	q	+++c	+++c	
b	7	d	+++c	+++c	
	3	q	+++c	+++c	
	3	q	+++c	+++c	
f	8	d	+++c	+++c	
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	73	q	+++c	+++c	+++n
f	3	q	+++c	+++c	+++n
	9	q	+++c	+++c	+++n
	9	d	+++c	+++c	+++n
x	6	q	+++c	+++c	+++n
	35	q	+++c	+++c	+++n
	31	q	+++c	+++c	+++n
s	0	q	+++c	+++c	+++n
	3	q	+++c	+++c	+++n
	3	q	+++c	+++c	+++n
<i>n a l h s a l o g</i>					
d	35	q	+++c	+++c	+++n
	44	q	+++c	+++c	+++n
	55	q	+++c	+++c	+++n
f	30	q	+++c	+++c	+++n

though cl n c a l l y a r e l a p s n g t h y m o m a n o m o r p h o
a l y c l e a r t h y m o m a o u s t i s s u e w a s f o u n d

s a g e d 3-17 y e a r s m e a n 6.9) h a d s m l a r d s
t o n p a t t e r n s f o r t h e s e r e c e p t o r s (T a b l e I)
h e r t h e c o r t e x n o r t h e m e d u l l a o f t h e s e
t u s e s s h o w e d r e c e p t o r s f o r F c y C 3 d o r C 3 b
v e t h y m u s b o p e s f r o m M G p a t i e n t s w e r e
p e d a c c o r d n g t o h i s t o p a t h o l o g y i n 5 c a s e s w i t h
t o m a (a g e d 30-63 m e a n 43) 13 w i t h f o l l i c u l a r



F g 7 M G t h y m u s s h o w n g a s t r o n g b n d n g o f C 3 - c o a t e d
o x e r y t h r o c y t e s o v e r n o d u l a r a r e a s i n t h e t h y m c m e d u l l a

h y p e r p l a s i a (a g e d 70-35 m e a n 76) a n d 4 w i t h n o r
m a l i n v o l u t e d m o r p h o l o g y w i t h o u t s g n s o f h y
p e r p l a s i a o r t h y m o m a (a g e d 30-55 m e a n 36)

I n t h r e e o f t h e f i v e t h y m o m a c a s e s t h e r e w a s n
d f u s e o v e r l a p p n g d s t r b u t o n o f r e c e p t o r s f o r
b o t h S E a n d F c y b u t n o C 3 r e c e p t o r s O n e c a s e
h a d n o S E r e c e p t o r s b u t a d f u s e o v e r l a p p n g d i s
t r b u t o n o f b o t h F c y a n d C 3 d r e c e p t o r s O n e c a s e
(n o 15) a l t h o u g h c l n c a l l y a n d r a d i o l o g c a l l y a r e
l a p s n g t h y m o m a h a d m o r p h o l o g c a l l y o n l y a r e a s
o f f o l l i c u l a r h y p e r p l a s i a a n d s h o w e d a r e c e p t o r n
d c a t i o n p a t t e r n c h a r a c t e r i s t i c o f t h i s k n d o f
t h y m u s m o r p h o l o g y (s e e b e l o w) w i t h c o r t c a l S E
r e c e p t o r s a n d m e d u l l a r y n o d u l e s p o s i t v e f o r C 3 d
r e c e p t o r s (T a b l e I)

A l l 13 m y a s t h e n c p a t i e n t s w i t h f o l l u l a r h y
p e r p l a s i a h a d r e c e p t o r s f o r S E A E T i n t h e c o r t c a l
a r e a s T w e l v e o f t h e s e c a s e s a l s o h a d a m o d e r
a t e l y s t r o n g F c y r e c e p t o r a c t i v i t y o v e r l a p p n g w i t h
S E r e c e p t o r s i n t h e c o r t e x s m l a r t o t h a t s e e n i n
t h e c o n t r o l s I n c o n t r a s t t o t h e f i n d n g s i n c o n t r o l
t h y m u s e s t h e s e M G t h y m u s e s h a d C 3 r e c e p t o r s
w i t h n n o d u l a r a r e a s i n t h e m e d u l l a (F i g 7) T h s
w a s s e e n i n a l l t h e s e c a s e s b u t t h e n u m b e r o f p o s i
t v e n o d u l e s v a r i e d w i t h n w d e r a n g e s

I n f o u r m y a s t h e n c p a t i e n t s t h e t h y m u s m o r
p h o l o g y a p p e a r e d e s s e n t i a l l y n o r m a l o r a t r o p h c
w i t h o u t s g n s o f f o l l u l a r h y p e r p l a s i a o r t h y m o m a
A l t h o u g h t h e r e w a s n o h i s t o l o g c a l l y e v d e n t f o l l u
l a r h y p e r p l a s i a t h e s e c a s e s i n a d d i t o n t o c o r t c a l
S E a n d F c y r e c e p t o r s a l s o h a d n o d u l a r C 3 r e c e p t o r
a c t i v i t y i n t h e t h y m c m e d u l l a (T a b l e I) q u i t e s m l a r
t o t h e r e c e p t o r d i s t r b u t o n p a t t e r n s e e n i n c a s e s
w i t h f o l l u l a r m e d u l l a r y h y p e r p l a s i a

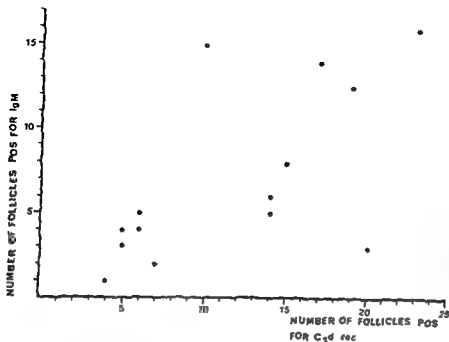


Fig 3. Relation between number of follicles for IgM by immunofluorescence and the number of follicles positive for C3d as compared on frozen sections.

The C3 receptor positive nodules in the thymic medulla of myasthenic patients were shown to be positive both for C3d and C3b receptors but to lack receptors for Fcγ, Fcμ and SE. In several cases the number of C3 receptor positive areas outnumbered the follicular areas detectable by morphology alone on serial sections. None of the myasthenic thymuses did bind OEAM indicator cells.

As expected around 90% of the thymic cells in suspension of either control or MG thymuses formed E rosettes; however relatively few cells formed Fcγ rosettes (Table II). This was also the case for Ig and C3d receptors (Table II). There was no statistically significant differences between the hyperplastic thymoma or control groups with regard to any of the markers studied (Table II). Only a few SE+ cells also expressed Fcγ receptors as indicated by concomitant double indication experiments.

Immunofluorescence

To further characterize the subpopulation of follicular cells in myasthenic thymuses positive for receptors, direct IFL studies were performed on serial sections to those used for C3 receptor immunofluorescence. Thirteen hyperplastic MG thymomas without thymoma and six control cases were investigated. By direct IFL on the cryostat sections the MG thymuses revealed Ig+ nodular areas in the medulla. None of the control thymuses were positive. The same nodular areas could be identified as positive both for C3 receptor and for Ig. A correlation was found between the number of areas positive for C3 receptors and Ig on serial sections (Fig 3). In general more C3 receptor positive areas were found than areas positive for Ig. With monospecific antisera the Ig+ areas were shown to contain both IgG and IgM as well as λ chains. Nodules positive for IgD were also found in several cases. The IgM staining was ex-

Table II. Surface markers expressed by thymic cells.

No of pats	Diagnosis	Percentage of cells with receptors for (mean and range)				
		SE-AET	Fcγ	Fcμ	C3	Ig
13	Control	90 (87-99)	7.4 (0-7)	<1	7.8 (0-11)	9.0
17	MG follicular hyperplasia	91.9 (8-97)	3.4 (0-9)	<1	5.3 (0-16)	3.8
6	MG thymoma	100 (79-97)	9 (7-10)	<1	3.5 (1-14)	6.8

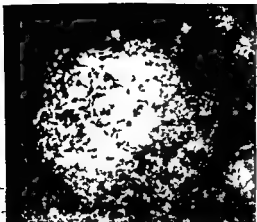


Fig. 4. MG thymus showing staining for IgM in nodular pattern of the thymic medulla. Note the lace-like membrane associated staining pattern and the more coarse granular staining of central areas.

present in the central part of the nodules but absent in the periphery whereas IgD predominant in the mantle zone of the nodules. Some positive cells in addition a few cells centrally located in the nodules were also IgD+ (Fig. 4).

Immunoperoxidase staining

The results of PAP staining corroborated in general the observations made by IFL and clearly evidenced the localization of Ig to cells of the nodular pattern.

Chemistry

The medullary nodules did not stain for ALP, ACP or peroxidase. Outside these areas there was only a weak reaction for both ACP and NSE in the medulla and a moderately positive reaction in the cortex. Only endothelial cells in capillaries and some reticular cells in follicles were positive for ALP. Peroxidase activity was usually found in a few reticular cells within the medullary nodules and in some cells associated with lobular areas. The latter cells were mostly identified as monocyte-like cells.

DISCUSSION

Previous characterization of thymocytes with reference to immunological markers have mainly been based on studies on resuspended thymus cells.

These studies have shown that the majority of thymocytes can bind SE but that some thymocytes do not (12).

By IFL usually less than 1% of the thymus cells are Ig+. However in MG an increased but varying number of Ig+ cells has been reported (Table II). Similar results were obtained in the present study with regard to SE+ and Ig+ cells in resuspended normal thymuses (Table III). In this study of MG thymuses we also observed an increased but variable percentage of Fcγ receptor positive cells. In addition the MG thymuses also contained variable numbers of cells with complement receptors. From Table III it is evident that the quantification of Ig bearing thymus cells in suspension gives quite variable results both between different investigators and from case to case. It seems that quantitative measurements on the frequency of B cells in resuspended MG thymuses might not be a very reliable method for the estimation of B cell activity.

Our observations on the histological distribution of receptors in cryosections of thymus tissue indicated that the majority of SE+ cells reside in the cortical area in agreement with the histologically clear dominance of thymocytes in these areas. The almost complete absence of SE binding in the medullary regions cannot be convincingly attributed to a lower concentration of thymocytes in these areas but is probably rather due to a lack of SE receptors with enough binding affinity to be demonstrated by the method used. The idea that medullary thymocytes have SE receptors with a weaker affinity is also supported by the observations that among resuspended thymocytes subpopulations with high and low affinity for SE can be found as estimated both by the number of SE bound to the thymocytes as well as varying capacity of thymocytes to bind SE at 37°C (12). Furthermore T cell areas in frozen sections of peripheral lymphoid tissues show virtually no SE binding although most resuspended T cells from peripheral lymphoid tissues form SE rosettes at room temperature or 4°C. These apparent differences in SE binding capacity between cortical and medullary thymocytes could reflect steps of thymocyte maturation within the thymus (13). In this respect medullary thymocytes seem to be more similar to peripheral T cells than cortical thymocytes.

On resuspended thymus cells Fcγ receptors have previously been shown to occur on a small thymocyte population (20). Our observations on

Table III Studies on the frequency of B and T cells in resuspended MG thymuses

Reference	No of pats	Diagnosis	Percentage of cells with receptor for (mean and range)				
			SE	Fcy	Fcμ	C3	Ig
Abdou et al (2)	4	MG	73			11	10
	4	Control	86			5	3
Shirai et al (22)	7	MG hyperplasia	89 (80-95)				<0.5
	2	Persistent thymoma	94 (92-97)				<0.5
	4	Control	95±3.4				<0.5
Birnbaum and Tsarnis (6)	5	MG	59.5 (35.9-88.6)			1.1 (0.8-1.6)	
	1	Thymoma	49.3			1.5	
	4	Control	(48.8-59.5)			(1.1-2.9)	
Cook et al (11)	12	MG	92				3
	6	Control	96				1
Lisak et al (15)	18	MG	79±1.6				19±1
	6	Control	89±4.5				9±
Aarli et al (1)	4	MG	90 (84-97)	7 (2-14)		17 ^a (25.9)	<1 ^b
	2	Thymoma	91 (90-92)	5 ^c (4-7)		14 (14-14)	1.5
	7	Control	86	6		6	<1
Present study	12	MG hyperplasia	91.9 (82-97)	3.4 (0-9)	<1	5.3 (6-16)	3.8 ^d (0-1)
	6	Persistent thymoma	88.3 (79-97)	9 (2-20)	<1	3.5 (1-14)	6.1 (0-2)
	13	Control	90 (82-99)	2.4 (0-7)	<1	2.8 (0-11)	2.9 (0-5)

^a Mean of two observations ^b One case tested ^c C3b ^d C3d

cryosections indicate an overlapping histological distribution of Fcy and SE receptors in cortical areas but it was not possible to conclude whether these two receptors were located on the same or different cells. However, the absence of Fcy indication in medullary regions of normal thymuses apparently excludes epithelial cells as having Fcy receptors. Furthermore, the experiments with simultaneous indication for SE and EAG receptors on cells in suspension seem to indicate that these receptors are mostly present on different thymic cells. Thus, cells that are not easily brought into suspension, i.e. stromal cells, may account for most of the cortical expression of Fcy receptors. Alternatively, these receptors are easier to indicate on sectioned thymic cells than on the surface of the resuspended cells.

The absence of demonstrable Fcμ and C3 receptors in sections of normal thymuses is in agreement

with observations on thymocytes in suspension (Table II).

In contrast to others (1) we found no difference in the SE receptor distribution pattern between MG and control thymuses. The same pattern of SE and Fcy receptors was found in both in the (young) control group and in MG patients. Thus, in this respect there are similarities between MG thymuses and the premalignant thymus of childhood and adolescence.

In the present study, receptors for C3 were found in all myasthenic patients except three who had thymomas. All MG patients with hyperplasia, normal or involuted thymic morphology had C3 receptors mostly concentrated in nodular areas in the thymic medulla. The number of C3+ nodules varied considerably between patients. Also thymus biopsies without histologically distinct nodules revealed C3 receptor-positive

ullary areas. Thus C3 receptor demonstration is more sensitive in revealing follicular/nodular hyperplasia than conventional histopathology. Similar observations of C3 receptors in follicular areas in MG thymuses have been reported elsewhere (1-4, 23). In agreement with Staber et al. (1) we found OEAMC binding selectively to the medullary follicles. In the other reports also a diffuse medullary binding was noted (1-4). These different patterns could be due to technical variations. In the present study bovine erythrocytes were used as indicator cells avoiding the binding of SE to E receptors. Occurrence of Ig+ cells in MG thymuses has been reported in several studies (Table 1).

Our morphological and immunologic findings (the presence of C3 receptors and Ig) support the conclusion that the nodular structures of the MG thymic medulla are follicular germinal centres equivalent to those containing proliferating B lymphocytes. The different distribution of IgM and IgD within these follicles seems to reflect stages in the differentiation of proliferating B lymphocytes identical to what we have seen in peripheral lymphoid tissue. Often numerous Ig+ and C3+ areas demonstrate in sections of MG thymuses usually compared with the relatively few B cells found in sections of the same MG thymuses. This indicates that the histological techniques were more sensitive in the demonstration of B cells than cell counting, probably due to difficulties in resuspending the B cells of the relatively cohesive follicular structures. We examined only relatively few cases of thymoma but some observations may have general implications. In all cases diffuse Fcγ receptor activity was seen histologically. In one case no SE receptors were found but a diffuse strong C3 reactivity suggesting that other lymphoid cells and thymocytes were associated with this thymoma. Thus our observations seem to indicate that thymomas are heterogeneous with respect to C3 receptors tested for. Since the examined thymomas were mixed proliferations of epithelial and lymphoid cells it was difficult to ascertain the type of cell expressed respective receptor activity. Obviously a larger spectrum of thymomas should be examined to further elucidate the immunological phenotype of the associated lymphoid cells.

The absence of B cell follicles and signs of Ig production within thymomatous tissue was a con-

sistent finding. This observation might be added to the clinical observations such as thymectomy response, age and sex distribution as well as anti-acetylcholine antibody titres suggesting that the thymoma cases are a disease entity somewhat different from other MG cases.

In conclusion, the present observations seem to indicate that high avidity SE+ thymocytes and OEAG+ thymus cells are concentrated mainly within the thymic cortex. In contrast thymocytes in medullary regions probably have receptors with lower binding avidity for SE and OEAG, if any.

In agreement with other histochemical studies (25) the medullary follicles showed staining reactions similar to those of lymph nodes (21) and excluded the possibility of monocytes being the cells responsible for the observed CR+ and Ig+ reactions.

In myasthenic thymuses nodular areas of the medulla corresponded to follicular B cell areas of peripheral lymphoid tissues in agreement with previous reports (1-23).

It is of interest from a diagnostic point of view to note that the histological demonstration of C3 receptors and Ig+ cells appeared to be more sensitive for demonstration of follicular areas in MG thymuses than conventional histopathology. This might be of clinical importance since a low number of follicles has been suggested to correlate with a better post-operative prognosis (3).

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High Immune Responsiveness in a Family with Multiple Paraproteinaemia and Autoimmune Thyroid Disease

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TRACT Immune responsiveness was investigated in a family comprising 12 first and second degree relatives one of whom had κ myelomatosis IgA paraproteinaemia two Graves disease and a further two thyroid antibodies without myeloma. Relatives by marriage served as controls. Parameters of immune capacity studied were the humoral and cellular immune response to haemolysin of *Helix pomatia* (HPH) dinitrochlorobenzene (DNCB) skin reactivity and in vitro lymphocyte proliferation capacity to phytohaemagglutinin (PHA). Mean antibody titres to HPH were higher in the family than in the control group in all main isotypes and IgG subclasses after primary and secondary immunization, and the difference was statistically significant for IgG. IgG and IgG₁ titres could not have been predicted from the (normal) serum Ig levels in this family. In vitro lymphocyte proliferation capacity to HPH after primary and secondary immunization was also significantly increased. DNCB skin reactivity also tended to be higher in the family whereas PHA induced lymphocyte proliferation was normal. These findings support the hypothesis that myelomatosis clusters in families with immune dysregulation.

Key words: paraproteinaemia thyroid disease familial immune response

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Genetic susceptibility to plasma cell dyscrasia is suggested in humans by a higher incidence of multiple myeloma (13, 15, 38) and macroglobulinemia (22) in relatives of patients with these diseases than in the general population. Some relatives (13, 22) but not all (26) have shown higher serum Ig levels in these relatives than in control populations and this might be interpreted as a tendency towards dysregulation of immunoglobulin (Ig) synthesis in these families. Further, as has been shown that strains of mice with easy

inducible plasma cell tumours have higher antibody responses than strains which do not develop plasma cell tumours (39).

Therefore we were interested in measuring the immune responsiveness in a family with paraproteinaemia. This was possible in all 10 living first and second-degree relatives of two brothers with κ myeloma and IgA paraproteinaemia respectively. The humoral immune responsiveness to the primary antigen haemocyanin of *Helix pomatia* (HPH) (14, 34) could be extensively measured by differentiating the antibody response in the IgM, IgG, IgA class specific and the four IgG subclass specific antibody titres to HPH after primary and secondary immunization (35). Cellular immune capacity was investigated by measuring in vitro lymphocyte proliferation capacity to HPH and to phytohaemagglutinin (PHA). Further dinitrochlorobenzene (DNCB) skin reactivity was measured being a sensitive in vivo parameter of cellular immune capacity. The results showed a tendency towards high immune responsiveness in this family compared to the relatives by marriage who served as a control group.

It appeared that also multiple autoimmune thyroid disease was present in this family in the form of Graves disease. An association between thyroid disease and paraproteinaemia has incidentally been described (2, 27, 33). It is of interest that also in autoimmune thyroid disease dysregulation of immune responsiveness has been suggested to be a pathogenetic factor (8).

STUDY POPULATION AND METHODS

Family. The family relationship is shown in Fig. 1. The parents of the first generation were deceased. There were

Abbreviations: Ig = immunoglobulin HPH = haemocyanin of *Helix pomatia* PHA = phytohaemagglutinin DNCB = dinitrochlorobenzene

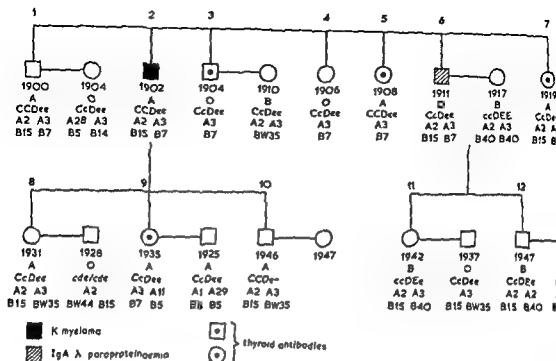


Fig 1 Relationships, year of birth, blood groups and HLA types of family members (numbered) and controls (relatives by marriage)

no known consanguinity and no indications of malignant paraproteinaemia or thyroid disease in these parents or the grandparents. In one of four brothers in the first generation line a x myeloma was diagnosed half a year before this study after a period of backpain and a spontaneous fracture of the fibula (referred to us by P. Blanksma and O. de Vries, Hospital Tjongerschans, Heerenveen). He has since then been treated with six weekly courses of melphalan and prednisone. He felt well at the time of study and had no complaints. Sternal aspirate still showed preponderance of myeloma cells which had only x light chain determinants in the cytoplasm by immunofluorescence. Immunoelectrophoresis of concentrated urine revealed x light chain proteinuria. Serum Ig levels were low (Table 1). Immunological studies were carried out during three weeks before a new cytostatic course.

IgA λ paraproteinaemia and otherwise normal serum Ig levels were found in another brother (Table 1). There was a slight increase in IgA λ containing plasma cells in the bone marrow. No Bence Jones proteinuria was detected by immunoelectrophoresis of 40 times concentrated urine. Bone X-ray studies were normal. He appeared healthy and had no complaints.

The youngest sister (no. 7) was a lively woman with a vascular bruit over a slight struma and a tremor of the hands but without eye symptoms. She had no complaints, appeared otherwise healthy and had a normal weight. Thyroid hyperfunction was confirmed by a T_4 serum level of 17.4 µg/100 ml (normal 7.0–12.5). Serum thyroid antibodies were present as shown by strongly positive immunofluorescence of her serum against thyroid cytoplas-

ma (presumably microsomal antibodies) (J. A. 1 Regional Public Health Laboratory, Groningen). Immunological studies were completed before treatment.

One sister (no. 5) had undergone a partial thyroidectomy 20 years earlier because of thyroid hyperfunction. She and another brother (no. 3) had weakly positive thyroid (cytoplasmic) antibodies without signs of hyper- or hypofunction. One brother and one sister had no abnormalities.

Five children of the two brothers with paraproteinaemia were also investigated. One daughter of the patient with x myeloma had weakly positive serum thyroid (cytoplasmic) antibodies. In all family members other serum antibodies were negative (rheumatoid factor, antinuclear, antimitochondrial and antismooth muscle antibodies).

Available relatives by marriage of the family were without detectable disease and served as controls (Fig. 1).

HLA and blood group typing of family members and controls (Fig. 1) was carried out by conventional techniques (J. M. van der Voort, Beelen and J. M. Stuyte, Steyn).

Serum Ig levels were determined by radioimmunoassay. IgG subclass measurements (T. Out, Laboratory for the Blood Transfusion Service, Amsterdam) were performed by a slightly modified technique according to van der Griend et al. (16). Results are expressed as percentages of standard serum containing 6.1 mg IgG, 3.2 mg IgG₁, 0.5 mg IgG₂, and 0.2 mg IgG₃ per ml.

Comparison of patients, family members and controls

	κ myeloma	IgA para- proteinaemia	Family members	Controls
	74	65	Mean and range 50.2 (29-76)	48.9 (26-72)
(mg/100 ml)				
	473	1397	1479 (924-1727)	1369 (979-1639)
	10	234	172 (70-312)	246 (107-822)
	7	784	204 (70-357)	236 (113-414)
(% of standard serum)				
	25	130	149 (105-205)	139 (90-155)
	70	200	186 (50-320)	181 (105-250)
	25	130	147 (80-270)	139 (100-215)
	90	85	96 (30-200)	65 (35-130)
at 3 weeks)				
	8	8	9.1 (6-11)	7.1 (3-10)
	4	9	7.4 (4-11)	5.3 (3-7)
	2	9	7.4 (6-10)	5.7 (4-9)
	3	6	7.6 (6-10)	6.0 (1-9)
	5	7	7.1 (6-9)	4.8 (1-8)
	3	6	5.8 (4-7)	4.6 (0-10)
	2	5	5.9 (5-8)	3.7 (1-5)
mutation index			Median and range	
I	1.2	2.9	1.2 (0.6-1.6)	1.1 (0.6-2.4)
II	3.8	2.8	16.7 (2.5-28.9)	5.0 (1.4-7.9)
III	-	3.0	22.8 (8.8-147.8)	6.7 (4.3-12.3)
mutation (dpm \times 1000)	37	24	70.7 (20.4-80.2)	37.9 (24.7-80.3)
score (maximal range 0-12)	12	12	12 (3-12)	11 (1-12)
Subjects			8-10	7-8

Humoral immune response Immunization was done with primary immunogen HPH (14/34) 1 mg subcutaneously at weeks 0 and III. Sera were collected at 2, 3, 6, 8 and 14. IgM, IgG, IgA class specific and IgG subclass specific anti-HPH titres were determined by an enzyme linked immunosorbent assay (ELISA) as previously described (34, 35). Titres are expressed as the number of doubling serum dilutions starting from 1:10 for IgM and IgA and with 1:20 = 0 for IgG subclasses of IgG.

Cellular immune response DTH skin reactivity was determined semiquantitatively by patch testing after sensitization as previously described (4). The minimal score was maximal 12. HPH induced lymphocyte transformation (6-day cultures, ^3H thymidine incorporation) before and after immunization was done as previously described (14, 36). Findings are expressed as a stimulation index which is the ratio between the cultures with and without antigen. PHA induced lymphocyte transformation (6-day cultures, ^3H thymidine incorporation) experiments were done as previously described.

Statistical analysis was done using Student's *t* test (unpaired) for Ig and titre comparisons and Wilcoxon's non-parametric test (two-tailed) for comparison of indices of humoral immunity. Significant statistical difference was defined at $p < 0.05$.

RESULTS

Humoral immune capacity

Serum Ig levels of family members did not differ from those of the controls (Table I) ($p > 0.20$ for all classes and IgG subclasses). **Ig class specific and IgG subclass specific anti-HPH titres** after primary and secondary immunization in family members without paraproteinaemia ($n=8$) were compared with those in the controls ($n=7$) (Figs 2 and 3). Mean values for all classes and subclasses were higher in the family at all points of time. The difference was statistically significant at most points of time for IgG, IgG₂ and IgG₄ anti-HPH titres (p values depicted in Figs 2 and 3). Anti-HPH titres three weeks after primary immunization in the two brothers with paraproteinaemia are compared with titres in the family and the control group in Table I. Values in the patient with IgA paraproteinaemia were within the range of his family. Values in the patient with κ myeloma were lower and within the range of the controls. The values of 4

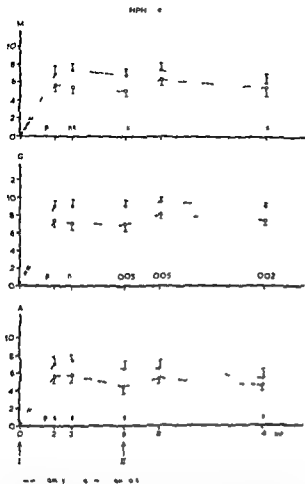


Fig. 2 IgM, IgG and IgA specific anti-HPH titres (mean \pm S.E.M.) after primary (I) and secondary immunization (II) in 8 family members (●) and 7 controls (○). Titres are expressed as titre steps represent ng double serum dilutions. P values above the various points of time indicate statistical significance (ns = not significant).

family members with thyroid antibodies did not differ from those of the other 4.

Cellular immune capacity

In vitro HPH induced lymphocyte transformation after immunization was significantly higher in the family group than in the control group after primary as well as secondary immunization (Table 1). P values were <0.05 at three weeks and <0.01 at eight weeks. There was a wide variation of HPH induced lymphocyte transformation indices in the family group varying from the narrow range present in the control group to high values. Although in the family as a group this higher *in vitro* cellular reactivity to HPH was associated with higher anti-HPH titres

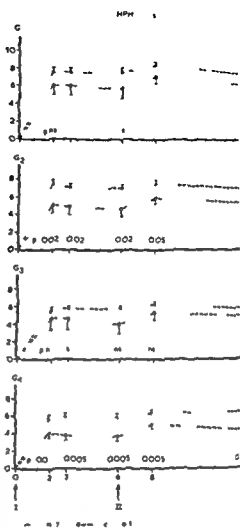


Fig. 3 IgG specific anti-HPH titres in 8 family members and controls. Same presentation as in Fig. 2.

in vivo there was no direct correlation between individual titres of any Ig class or subclass and values of *in vitro* lymphocyte stimulation. Family members with signs of autoimmune thyroid disease did not differ from the others. Both brothers with parathyroidism had values within the range of controls (Table 1).

DNCB skin reactivity score (Table 1). The skin score was higher in the family than in the control group but the difference was not significant ($0.05 < p < 0.10$). Both patients with parathyroidism as well as the four family members with thyroid antibodies had score 12, the highest possible.

HPH induced lymphocyte reactivity
1) There was no difference between the 4

and the control group. Both patients with paraproteinaemia had normal values.

COMMENTS

Parameters of immune capacity were studied in a family comprising 12 first and second-degree relatives.

Two family members had paraproteinaemia (malignant); two had active autoimmune disease (Graves' disease)—one at the time of study and one in the past—and a further had weakly positive thyroid antibodies without disease.

The humoral immune response to a primary test antigen (HPH) was higher in this family than in the controls who were relatives by marriage of the family members. This result could not have been predicted on the basis of the (normal) serum IgG levels in the family. Considering *in vitro* antigen-induced lymphocyte transformation as a sensitive index of cellular immune capacity (15), this family showed also cellular hyperimmune responsiveness to haemocyanin. Of the other measured parameters of cellular immune capacity, DNCB reactivity also tended to be high whereas PHA-induced lymphocyte proliferation was normal. Induced lymphocyte proliferation capacity is probably less suitable to measure hyperimmune responsiveness because it is already a very strong stimulant at optimal dosage in normals.

The findings in this family show a certain resemblance to the situation in Balb/c mice which exhibit humoral hyperimmune responsiveness together with easily inducible plasma cell tumours. Dysregulation of Ig synthesis has been observed in families with myeloma and paraproteinaemia on the basis of higher serum Ig levels in control populations (13, 22). In the family described here serum Ig levels were not clearly elevated. However, humoral immune responsiveness towards antigens is not necessarily related with high Ig levels. The genetic control of humoral immune responsiveness in man is probably on two levels: general via the rate of Ig synthesis and more specific dependent on antigen structure (24). Relations between serum Ig levels and specific immune responses have to our knowledge not been investigated in human families but Ig class specific immune responses to haemocyanin did not correlate with serum Ig levels in non-related normals (35).

Hyperimmune responsiveness or a hyperactive

Ig-producing system or immune stimulation is probably not enough to make the subject more prone to develop a plasma cell tumour. Transition from polyclonal to monoclonal hypergamma globulinaemia has been observed in patients with rheumatoid arthritis, cirrhosis of the liver (9, 40) and in animal models of autoimmunity (28, 29). However, in man the incidence of plasma cell tumours in these diseases is not clearly increased (10, 19). A relation was found between myeloma and pernicious anaemia (23) but pernicious anaemia is associated with hypo- and not with hypergamma globulinaemia (17). The second factor which seems necessary for the development of a plasma cell tumour is a susceptibility to oncogenesis of plasma cells (30, 31).

Autoimmune thyroid disease includes Graves' disease (8). Thyroid stimulating antibodies play a role together with diagnostically important thyroid-specific microsomal and thyroglobulin antibodies. An immune suppressor cell defect leading to uncontrolled immune responses towards thyroid tissue has been suggested (1, 8).

It is tempting to suggest the presence of a general immune suppressor cell defect (32) in the family presented here on the basis of increased responses towards humoral and cellular test immunogens *in vivo* and *in vitro*. Such a defect by leading to uncontrolled immune reactions to antigens including self-antigens might explain the multiple occurrence of paraproteinaemia and thyroid disease in one generation of this family.

HLA B7 and B5 were present in this family. This is different from observed associations in Graves' disease (B8) (18, 21), myeloma (B5, B18) (3, 12) and partly different from observations in benign paraproteinaemia (increased frequency of B7 and decreased frequency of B15) (6). Immune responsiveness in man is probably related to the major histocompatibility complex as in mice but a clear relationship between HLA types and immune responsiveness was not apparent (24).

It is concluded that humoral hyperimmune responsiveness in certain instances may be an important prerequisite for the development of human plasma cell tumours as is the case in the animal plasma cell tumour model. Both patients with paraproteinaemia in this family showed less signs of hyperimmune responsiveness and this might be explained by the fact that malignant and sometimes benign plasma cell tumours themselves depress

humoral and cellular immunity (5 7 11 20 35 36 37)

ADDENDUM

A third case of paraproteinaemia was diagnosed 3 years later in a sister of the two brothers with paraproteinaemia (no 3). She had a history of thyroid hyperfunction and had been well until recently when she was admitted to a hospital because of an acute major hemiplegia. Cerebrovascular thrombosis was diagnosed. Routine laboratory tests revealed no abnormalities. X-ray of the chest was normal. She was transferred to a nursing home and died of pneumonia and cardiac decompensation within 2 weeks. A blood test shortly before death revealed no cryoglobulinaemia or paracoagulation. Serum electrophoresis disclosed two faint bands in the beta gamma region. Subsequent immunoelectrophoresis showed a moderate amount of Bence Jones λ protein. Urine could not be studied with immunoelectrophoresis but an Albustix test had been negative. This does not exclude the presence of a significant Bence Jones proteinuria. Serum Ig levels were IgG 720 IgA 60 and IgM 30 mg/100 ml. ESR was 13 mm/hour blood Hb 15.6 g/100 ml blood urea 5.5 mmol/l serum creatinine 85 μ mol/l and total serum protein 6.3 g/100 ml. Serum thyroxine level was normal.

Re-examination of serum and urine stored 3 years earlier disclosed no abnormal bands on electrophoresis but immunoelectrophoresis with a panel of different Bence Jones antisera showed that a slight but distinct Bence Jones λ paraproteinaemia and proteinuria had been missed at that time by the antisera in use. Serum Ig levels at that time were IgG 924 IgA 70 and IgM 70 mg/100 ml.

This patient manifested in 3 years a slowly increasing Bence Jones paraproteinaemia without overt signs of myelomatosis at the time of death. She links in her person the thyroid disease and paraproteinaemia which were both multiple but separately present in her relatives. Parameters of immune capacity in this patient 3 years before death of probably arteriosclerotic cerebrovascular disease were excellent as compared to the controls and intermediate in the higher range observed in her family (CHPH titres 3 weeks after immunization G7 M7 A6 G₁ 7 G₂ 5 G₄ 5 HPH stimulation index 0 weeks 1 2 3 weeks 5 5 8 weeks 18 8 PHA 28800 DNCB 9) (Table I).

ACKNOWLEDGEMENT

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Demonstration and Partial Characterization of an Atypical Protein in the Urine of a Patient with Primary Amyloidosis

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ABSTRACT A paraprotein has been isolated from the urine of a patient with primary amyloidosis. Immunologically it was classified as a free λ light chain. Molecular weight was 22 500 daltons. N-terminal amino acid analysis demonstrated homology with variable subgroup in 19 of the first 20 amino acids. Extensive homology with λ IV chains was demonstrated also in the hypervariable region of the light chain. An antiserum produced against the protein was rendered idiotype specific by absorption with pooled human light chains. This antiserum stained tissue specimen from the rectum and the patient by the indirect immunofluorescence technique. This strongly indicates that the free light chains that can be isolated from the urine are deposited in the tissues as amyloid substance.

Key words: amino acid sequence, immunohistochemistry, γ amyloidosis, urinary amyloid protein.

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Classification of amyloidosis is rather complex and confusing. From the clinical point of view, amyloidosis has been divided into three major classes: 1) Primary amyloidosis without detectable underlying disorder; 2) Secondary amyloidosis that is associated with systemic diseases or chronic infection; 3) A form of amyloidosis associated with hematosis (3). From the histochemical point of view, amyloidosis can also be classified according to the site of amyloid deposits in the walls of the blood vessels. The perireticular amyloidosis is usually limited to the intimal layer of the blood vessels, whereas the pericollagen amyloidosis is located to adventitial layers of the vascular bed. Amyloid substance deposits in tissues can be demonstrated

after Congo red staining giving a green birefringence (18).

The amyloid substance has been defined as extracellular material which is composed mainly of proteins with a fibrillar structure. X-ray crystallography has shown that the fibrillar material is arranged in a β pleated structure. The solubilization of these fibril proteins has rendered them accessible to investigation by biochemical techniques. Until now, three major classes of amyloid fibril proteins have been characterized.

1. A group of proteins with varying molecular weight from about 5000 to 9000 daltons (12, 18). The origin of these proteins is unknown; they are unrelated to immunoglobulins and are termed protein AA (26). Protein AA shows antigenic cross reaction with a serum α_1 globulin which has been regarded as a precursor protein of the amyloid fibril protein AA (26). Studies have further shown that protein AA is the major component in the amyloid fibrils from patients with secondary amyloidosis.

2. The second class of amyloid fibril proteins consists of immunoglobulin light chains or light chain fragments (AIO) (18). AIO proteins have mostly been seen in amyloid fibrils of patients with primary or myeloma associated amyloidosis. So far, only two amyloid related urinary proteins, one κ and one λ chain, have been extensively studied (26).

3. Evidence has been presented supporting the

Abbreviations: PAGE - polyacrylamide gel electrophoresis; SDS - sodium dodecyl sulfate; DI - difference index; ELISA - enzyme linked immunosorbent assay; OD - optical density; PBS - phosphate buffered saline.

hypothesis that the amyloid P component (protein AP) also is an integral part of the amyloid fibril (7).

We have isolated a free light chain from the urine of a patient with a well established primary amyloidosis. It is the aim of this report to characterize further this protein both immunologically and with respect to its amino acid sequences.

CASE REPORT

A man born in 1916 was admitted to the Department of Medicine, Østfold County Hospital, because of dyspnea during exercise and pretibial edema. He also complained of bowel symptoms including constipation and periodic arthralgia, anorexia and loss of initiative.

Physical examination revealed markedly hypertrophic parotides, enlarged submandibular glands and macroglossia. The skin and nails were dry and atrophied. The heart was large with a relative cardiac volume exceeding the upper limit of the range of reference. No cardiac murmurs were heard.

The blood pressure was normal (100/60). Hb 11.5 g/100 ml, ESR 77 mm/h, RBC $4.7 \times 10^{12}/l$, WBC $5.6 \times 10^9/l$ and platelets $190 \times 10^9/l$. Repeated peripheral blood smears were all normal. Bone marrow aspiration specimens contained on several occasions less than 5% plasma cells. Serum iron and total iron binding capacity, serum vitamin B₁₂ and folate acid as well as thyroid function tests included T₃, T₄, TSH and TRH were within the range of reference. Liver function tests and liver enzymes including ASAT, ALAT, γ -GT and ALP were all normal. No protein anomalies were demonstrated by routine electrophoresis on cellulose acetate. Nor was any M component demonstrated by agarose gel electrophoresis of serum.

The patient's renal function was normal as evaluated by creatinine and creatinine clearance. Electrolytes, serum triglycerides, cholesterol and lipoproteins were also normal. Electrophoresis of concentrated urine, however, disclosed a mixed proteinuria including albumin and a paraprotein of light chain nature (0.7 g/l). Endocrine pancreas insufficiency could not be demonstrated as fasting blood glucose and a glucose tolerance test were within the range of reference. His exocrine pancreatic function was not tested. Antinuclear factor, Waaler and Ra Latex tests were negative.

X-ray studies of colon demonstrated small diverticula in the sigmoid part. X-ray studies of the skeleton did not reveal osteolytic changes or osteoporosis. However, small changes due to osteoarthritis were detected in the left hip. Scintiscans of the skeleton were normal as well as scintiphotos of liver and spleen.

Biopsy of the temporal artery was normal. Biopsies of rectum, skin and liver, however, demonstrated amyloid infiltration located to the vascular walls but also with small infiltration of the adventitial regions. Biopsy of systemic disease, chronic infection or myelomatosis were detected. The diagnosis of primary amyloidosis was made. No treatment except regular cardiac insufficiency regimen had been instituted.

MATERIALS AND METHOD

Serum was taken from the patient by regular puncture. Urine was collected on several occasions. 24-hour collections.

Electrophoresis of serum, urine and the various

obtained after column separation was performed in 1% agarose gel as described by Johanson. On some studies were performed by polyacrylamide electrophoresis (PAGE) mainly in test tubes. Sodium dodecyl sulfate (SDS)-PAGE were performed to estimate the molecular weight of amyloid protein (8). The immunological method used was the conventional double diffusion according to Ouchterlony (17). (A part of the material obtained from Dacopatts and Behringwerke.)

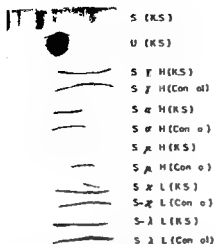
Isolation of the free light chain from the

The paraprotein was isolated from the urine in different ways. In order to remove albumin, concentrated column affinity chromatography with a 4B Blue Dextran conjugate as originally described by Travis and Fannell (25) but with some modifications (29). Urine first dialyzed against saline (0.9%) was applied to the column. One ml of concentrate was applied to the column for each run. The eluted in two steps, namely with 0.1 M Tris-HCl and 6 M urea. The eluates were then concentrated by dialysis against saline in vacuum. In this step HCl eluate contained the amyloid protein. The eluate contained the albumin as evidenced by electrophoresis in agarose gel demonstration of band. Immunoelectrophoresis of the concentrate, HCl eluate against anti λ and anti κ sera (30) demonstrated that the amyloid protein was in nature. A faint κ reaction was obtained occasionally.

In some experiments the urine was dialyzed against distilled water and lyophilized. The lyophilized material was dissolved in 0.05 M Tris-HCl (pH 7.6) and applied to a Sephadex G 700 column. A low molecular weight fraction (approximately 100,000 daltons) of light chains was recovered. The protein recovered fraction was approximately 0.75 g/l. It was demonstrated one single band in agarose gel. Immunologically the fraction contained mainly peptide chains but traces of κ chains were also demonstrated. κ chains were removed by applying the whole fraction on an immunosorbent column containing immobilized rabbit anti human κ light chain antibodies. The purified fraction gave no reaction against κ light chain in immunoelectrophoresis. This protein fraction demonstrated one band in PAGE.

Studies on the amino acid

The purified amyloid protein was subjected to studies including analysis of total amino acid composition determined on a BOCAL BC 700 amino acid analyzer connected to an Autolab Computing Integrator 5 system (AA) after acid hydrolysis of the samples for 74 hours (22, 23).



Agarose gel electrophoresis of serum (S) and urine of the patient (K) showing normal serum patterns and the urinary excretion of a paraprotein classed as immunoelectrophoresis as lambda light chains.

amino acid relatedness of the isolated free lambda light with other lambda and kappa light chains was studied after titration of the difference index (DI) of Metzger et al.

Immunological sequence analysis

Immunological analysis was performed with a JAS-47K JEOL Amino Acid Analyzer using 0.1 M Quad of buffer with 1.0 nmols were applied to the analyzer. The amino acid analysis was analysed on thin layer chromatography and gas chromatography (13).

Immunization

Immunization was raised against the purified free lambda light chain in a rabbit. The antiserum was rendered isotopic after absorption on two immunosorbent columns (Sepharose 4B) to which were coupled light chains isolated from pooled human IgG and human serum respectively.

Immunoelectrophoresis (ELISA) for testing of the specificity of the antiserum

Immunoelectrophoresis was performed in disposable polystyrene tubes (IC N1007 Denmark). All incubations were performed at room temperature. The tubes were coated by a solution for two hours with 10 ml of a solution of a pooled light chain and isolated Bence Jones lambda chains in a coating buffer consisting of 0.05 M sodium buffer pH 9.6 with 0.05% Na₂S₂O₃. After washing the tubes with 0.9% NaCl with 0.05% Tween 20, the tubes were incubated for 6-8 hours with 0.1 ml of an anti-phosphate buffered saline (PBS) with 0.05% Na₂S₂O₃ and 0.05% NaN. After this incubation the tubes were washed as described above and incubated with 100 μl of swine antirabbit IgG antiserum conjugated with alkaline phosphatase (Orion Finland). The conjugate was

used in a dilution of 1/500 with PBS containing 0.05% Tween 20 and 0.05% Na₂S₂O₃. The incubation time was 10-15 hours.

After subsequent washing as described above 1.0 ml of the substrate solution p-nitrophenylphosphate (1 mg/ml in 1 M diethanolamine HCl buffer pH 9.8) was added to the tubes and the enzymatic reaction was stopped by adding 100 μl of 5 M NaOH to the tubes. The reaction was stopped before the solution had an optical density (OD) 1 at 400 nm and the OD₄₀₀ measured was referred to a standard time 100 min assuming a linear reaction rate.

Immunohistochemical studies

Sections from rectum and ileocecal regions were cut on a microtome. After washing in PBS the sections were permitted to react with the antiserum at room temperature for 30 min. After a new wash in PBS the sections were reacted with a FITC-conjugated goat antirabbit immunoglobulin antiserum (Behringwerke Marburg Lahn W. Germany) for 30 min at room temperature. After washing in PBS the sections were studied in a Leitz Orthoplan microscope with an Osram HBO-500 high pressure mercury vapor burner light source and a Leitz Vertical Illuminator. The studies were repeated after the antiserum and antiserum had been preabsorbed as described earlier (4). Sections from rectum and ileocecal regions from patients with other disorders than amyloidosis were stained with the same antiserum.

RESULTS

Electrophoretic and immunological studies

Agarose gel electrophoresis of serum from the patient was normal without any paraprotein (Fig. 1). Concentrated urine however disclosed paraprotein excretion in addition to small concentrations

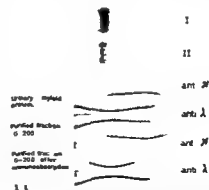


Fig. 2 Electrophoresis of freeze-dried amyloid protein from urine (I) after G 00 gel filtration (II). Immunoelectrophoresis demonstrates that the purified G 200 fraction after immunoadsorption with anti kappa serum contains lambda light chains only. The bottom well contains a lambda light chain control.

Table I Total amino acid composition of the patient's amyloid protein compared to the composition of lambda and kappa light chains

	Amyloid protein	Lambda L	Kappa L
Asp	20	14	20
Thr	21	21	19
Ser	26	31	27
Glu	29	21	26
Pro	16	15	11
Gly	15	15	13
Ala	15	18	12
I/2 Cys	5	5	5
Val	14	17	13
Met	2	1	1
Ile	5	5	9
Leu	14	13	15
Tyr	7	8	10
Phe	5	5	9
His	3	3	3
Lys	11	12	13
Arg	6	6	6
Glucosamine	3	—	—
Galactosamine	1	—	—

* The calculation is based on a total of 213 residues and a mol wt of about 23 000 daltons (Res/100 = 213)

of albumin and an unknown trace contamination of protein nature (Fig. 1). By immunoelectrophoresis the paraprotein had mainly λ specificities, but occasionally a positive κ reaction was seen (Fig. 1).

Fig. 2 shows the paraprotein preparation after purification on a G 200 column with subsequent column affinity chromatography, demonstrating one single band by electrophoresis in agarose gel and PAGE.

SDS-PAGE studies with marker proteins indicated a molecular weight close to 22 000 daltons.

Total amino acid analysis

Total amino acid analysis of the purified paraprotein preparation demonstrated a composition similar to λ light chain (Table I).

Calculation of the DI did not contribute significantly to the identification, since DI values of amyloid paraprotein/ λ L amyloid paraprotein/ κ L and λ L/ κ L all were very close to 10 (9.5, 10.5 and 10.5 respectively). Interestingly both galactosamine and glucosamine were detected in the amyloid protein (Table I).

Partial amino acid sequence analysis

Partial N-terminal amino acid sequence analysis of the first 31 amino acids of the amyloid protein was

Table II Test for anti-idiotypic specificity: adsorbed antiserum against the urinary λ L and other isolated human Ig light chains by ELISA method

The antiserum was used in a dilution of 1/100	
Coat (1 µg/ml)	OD ₄₉₂ /100 nm
Pooled light chains*	0.07
Urinary λ chain	1.63
HBJ, λ III*	0.08
HBJ, λ I*	0.06
HBJ, λ II*	0.03
Buffer	0.07

* Isolated from reduced and alkylated pooled Ig.

* Isolated human Bence Jones proteins with I subgroup.

Control experiments in which tubes with the size above were incubated with conjugate gave a result less than 0.1.

NH-Tyr-Glu¹ Leu² Thr³ Gln⁴ Pro-Pro-Ser⁵ Val⁶
Val⁷ Ser⁸ Pro-Gly⁹ Gln¹⁰-Thr¹¹ Ala¹² Ser¹³ Ile¹⁴ Thr¹⁵
(Ser)¹⁶-Gly¹⁷ Asp¹⁸-I-Leu¹⁹ Gly²⁰ Asp-Glu²¹ Tyr²² Glu²³

In position 25 no amino acid residue could be identified with certainty. Only one amino acid was detected in each position, except in position 25 where both isoleucine and leucine were detected. The amino acid sequence revealed that this is homologous to a λ IV immunoglobulin light chain (16). Among the 30 amino acid residues 26 are identical. The N-terminal sequence is also very similar to an AIO protein isolated from the spleen of a patient with primary amyloidosis (27).

Immunofluorescence staining of sections from rectum and liver biopsies

The antiserum was idiotype specific as it stained with the immunogen (the free urinary λ L chain) but not against pooled human light chains with different free λ light chains with different subgroups (Table II).

The sections from the rectum and liver of the patient stained specifically with the antiserum, indicating that the paraprotein demonstrated in the urine might be the same as the deposited amyloid substance in the tissues. Liver and liver biopsies from patients with other forms of amyloidosis did not stain positively with anti-idiotypic antiserum.

DISCUSSION

We have isolated an amyloid protein with a molecular weight of 22 500 daltons from the urine of a patient with primary amyloidosis. The protein has been purified and N terminal analyses revealed that it belongs to the λ IV subgroup.

When comparing this protein with the AIO fibril in 808 (26) the N terminal amino acid sequence up to position 20 is identical except for an exchange of aspartic to glutamic acid residue in position 2. The two other amyloid related Bence Jones proteins reported in (7,6) have a quite different N terminal amino acid sequence but both λ chains. However a tyrosine residue in position 30 has been suggested to be of special significance.

Another rather unusual feature of this Bence Jones protein is the content of glucosamine and tosylamine. Of the homogeneous light chains κ or λ type Bence Jones proteins or myeloma chains analysed only about 15% have been found to contain carbohydrate (6).

The relative contribution to the pathogenesis of amyloidosis by proteins of immunoglobulin nature and non immunoglobulin origin (viz AA) is under active investigation. In vitro studies have shown that several of these proteins will form fibrils with the properties of amyloid fibrils such as affinity for Congo red and green birefringence after such staining (12).

Wanner et al (5) gave the first proof by sequence analysis that some human amyloid fibrils are homologous in structure with κ light chains (κ I group). Terry et al (24) also have demonstrated similarity between urinary Bence Jones light chains (chains) and the predominant protein present in amyloid deposits. This Bence Jones protein (named Tew) was subsequently sequenced by Arn et al (19).

The anti idiotype antiserum used in this study showed no reactivity against pooled light chains and isolated human Bence Jones proteins of Va and Vb subgroup I, II and III. These results strongly indicate anti idiotypic specificity of the antiserum. By means of this specific antiserum and indirect immunofluorescence technique we were able to prove that the same light chains as were isolated from the urine of our patient is also deposited in tissues as amyloid substance. It is of interest that some light chains with certain variable groups tend to be associated with amyloidosis

closer than other light chains. Our study indicates that λ IV light chains are also amyloidogenic.

Amyloid deposition of light chains or light chain fragments have only been observed in man (12). Casein injections have long been used for experimental induction of amyloidosis in mice (12). It has been postulated that the endotoxin of *E. coli* which often contaminates casein may be the factor that causes induction (1).

Since one type of amyloidosis in man is related to immunoglobulin light chain deposition one might speculate whether amyloidosis is related to the T cell system alone or to disturbances in the T cell suppressor and helper cell systems which are involved in the regulation of B cell responses to certain antigens. Some investigators have reported on an increase in B cell proliferation of spleen cells in mice during and after amyloid deposition probably due to polyclonal B cell activation (21). The T lymphocyte responses to phytohemagglutinin both in man and mice have however shown no distinct abnormalities in two studies (2, 20) indicating normal T lymphocyte functions.

λ Light chains as observed in this study are probably more commonly associated with amyloidosis than κ chains (12).

Clinically the symptoms commonly associated with amyloidosis are fatigue, weight loss, ankle edema, dyspnea, parasthesias, hoarseness and occasionally gross bleeding. All these symptoms except bleeding were present in our patient. On physical examination especially the macroglossia and enlargement of the submandibular glands were extremely impressive.

The treatment of amyloidosis patients is far from satisfactory. Since at least part of the amyloid substance contains light chains or light chain fragments which are produced by plasma cells it has seemed reasonable to treat amyloidosis with alkylating agents that are effective against diseases characterized by proliferation of neoplastic plasma cells.

Two patients with primary amyloidosis have indeed benefited from such therapy (10, 14) with melphalan, prednisone and D-penicillamine. However a rapidly developing fatal leukemia has appeared during melphalan therapy in patients with amyloidosis (11). Our patient did not show any significant increase in plasma cells and we therefore decided not to institute any chemotherapy. This decision is in accordance with the recent double

blind study by Kyle and Greipp (13) who found no significant difference in survival rate between patients given placebo and amyloid patients treated with melphalan and prednisone.

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The Epidemiology of Thyrotoxicosis in Denmark

Incidence and Geographical Variation in the Funen Region 1972-1974

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ABSTRACT In a study based on a review of all thyroid function tests performed in the Funen region over a three year period, from Jan. 1, 1972 to Dec. 31, 1974, the annual incidence rates of thyrotoxicosis were found to be 46.5/100,000 for women and 8.7/100,000 for men. In both sexes the incidence rates rose with age. An analysis of the geographical distribution of the cases showed a significantly lower incidence rate among women from urban than from rural areas. It is estimated that the lifetime risk of developing thyrotoxicosis is 5% for women and 1% for men, and that the prevalence of existing and previously diagnosed thyrotoxicosis is 1.4% for Danish women and 0.3% for Danish men.

Key words: thyrotoxicosis, incidence, geographical variation.

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Thyrotoxicosis exhibits several characteristic epidemiological features with different incidence rates in both sexes and within different geographical areas. In addition, earlier studies have described marked rises in incidence (7) and have indicated a wide age distribution of new cases of thyrotoxicosis. The incidence has undergone certain variations (8-10). This study has been undertaken to give a current analysis of the epidemiology of thyrotoxicosis in the Danish population. Contrary to previous studies, the present survey also includes cases diagnosed in the hospitals.

STUDY POPULATION

The population studied was that of the Funen region, comprising approximately 450,000 inhabitants. They form a geographically representative sample of 9% of the Danish population (Green, unpublished paper). The study covered the period Jan. 1, 1972-Dec. 31, 1974.

The Funen region forms a geographically well defined administrative unit under one health authority. The Regional Hospital—where all specialties are represented—is situated in central Odense with 10 smaller hospitals in the surrounding area.

Endemic goitre does not occur in the Funen region. Demographic data have been obtained from Danmarks Statistik (3, 4, 5).

MATERIAL AND METHODS

During the study period, thyroid function tests were performed by the hospital laboratories in Svendborg and Odense, toward the end of the study exclusively by the Department of Clinical Chemistry, Odense Hospital. During a brief part of the study, one of the hospitals referred its thyroid function tests to a laboratory outside the Funen region.

The function tests comprised serum protein bound iodine, serum Thyroxine, T_4 -resin test and serum triiodine thyronine. Basal metabolic rate was estimated occasionally but not as a routine test.

When reviewing all the thyroid function tests performed throughout the three year period, we identified the patients with raised values, i.e. values above the normal reference range used by the laboratories. Additionally, patients from the hospital referring the tests to a laboratory outside Funen were separately investigated and included in the study if the criteria were fulfilled. Finally, basal metabolic rate tests performed were subjected to a special review in order to identify patients who had undergone this test only.

For each patient showing raised values, an enquiry was made to the referring physician or hospital department in order to check whether the diagnosis of thyrotoxicosis had been made and if so, to obtain the time of the first diagnosis and the type of treatment instituted. In a few cases, this information had to be requested from the patients themselves. In this way, a register was established of all newly diagnosed cases of thyrotoxicosis with treatment instituted in the Funen region over the period Jan. 1, 1972 to Dec. 31, 1974. No attempt has been made to divide the cases into subgroups such as Graves' disease, toxic adenoma, etc.

Analyses

The incidence of thyrotoxicosis is expressed as the average annual rates specified for age and sex. Standard errors

Table I *Newly diagnosed cases of thyrotoxicosis in the Funen region Denmark 1972-74*

Age group (y.)	Males				Females			
	No of cases 1972-74	Population*	Mean annual rate (per 10 ⁵)	1 S.E.	No of cases 1972-74	Population*	Mean annual rate (per 10 ⁵)	1 S.E.
0-9	~	34 617	0		-	32 585	0	
10-19	2	34 042	2.0	1.35	5	32 140	5.2	2.35
20-29	5	34 239	4.9	2.15	33	32 140	34.2	6.95
30-39	8	26 263	10.2	3.60	42	25 890	54.1	8.35
40-49	13	24 848	17.4	4.85	52	25 275	69.6	9.50
50-59	7	25 431	9.2	3.45	62	26 175	79.0	10.00
60-69	8	22 396	11.9	4.20	49	23 833	68.5	9.75
70-79	9	12 812	23.4	7.80	47	15 836	98.9	14.45
80+	5	4 813	34.6	15.50	17	6 429	88.1	21.40
Total	57	219 461	8.7	1.15	307	220 303	46.5	2.65

* Mean for each of the years 1972, 1973 and 1974.

(S.E.) of these rates are calculated from the approximate formula

$$\frac{1}{\sqrt{3}} \sqrt{\frac{pq}{n}}$$

in which p = the calculated three year rate $q = 1-p$ and n = the size of the age group (expressed as the average of the size in the three years of study)

In order to analyse the geographical distribution all cases of thyrotoxicosis have been distributed within three regions on the basis of their postal address at the time of diagnosis. Region I (city area) the municipality of Odense, region II (town area) the municipalities of Svendborg, Middelfart, Nyborg and Fåborg combined, region III (rural area) the rest of the total Funen region.

The population of each region has been standardized using the entire population of Funen as the standard. Comparisons between two standardized three year rates were made by calculating the standardized normal deviation (1) using a significance level of 5%. The cumulative morbidity risk has been calculated according to the principle of the life table method (2).

RESULTS

The study comprises 364 cases, 307 were men. In 323 (89%) of the cases the diagnosis was established during admission to a hospital and sex distribution was the same for those diagnosed in or outside hospital. In only one case was the diagnosis of thyrotoxicosis established on the basis of the treatment instituted on the basis of the metabolic rate test alone.

As no differences were found between the data for the years 1972, 1973 and 1974 the data for the whole period have been pooled for analysis. Table I shows the age and sex distribution of cases and the corresponding incidence rates. A significant difference is seen between the sexes, the overall incidence in women being 46.5 (1 S.E. = 2.65) per 100 000 and in men 8.7 (1 S.E. = 1.15) per 100 000. Distinct age variations are also seen in both

Table II *Geographical distribution of the cases*

Type of area	Area I (Odense)	Area II (Svendborg, Nyborg, Middelfart and Faaborg)	Area III (The rest of the County of Funen)
	City	Towns	Rural
Population density (inhabitants/km ²)	553	162	72
No. of males	81 805	43 313	94 345
No. of females	85 683	45 020	89 490
No. of thyrotoxicosis cases 1972-74	22 ♂ 104 ♀	11 ♂ 62 ♀	24 ♂ 141 ♀
Standardized 3-year incidence rate per 100 000*	27.7 ♂ 123.1 ♀	24.7 ♂ 132.8 ♀	23.5 ♂ 159.9 ♀

* Standard: the total population of the County of Funen.

* Standardized normal deviation = 2.01 (0.01 < p < 0.05).

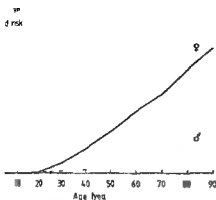


Fig. 1. Estimated cumulative risk of developing thyrotoxicosis.

the incidence rate rising progressively. The incidence rate in women (98.9/100 000) is highest in the group aged 70–79 and in men (34.6/100 000) in the group aged 80 and more with a small peak in the age group 40–49.

Table II shows the geographical distribution of thyrotoxicosis cases and the general standardized rates for each area in relation to both sexes. It appears that there is a tendency towards a higher incidence of thyrotoxicosis among women in areas with a lower degree of urbanisation. A comparison between area I with the highest and area II with the lowest degree of urbanisation shows a significant difference ($0.01 < p < 0.05$). None of the other differences are significant.

The age specific incidence rates have been used as the basis for calculation of the morbidity risk for both sexes (Fig. 1). In this connection the morbidity risk at a given age is defined as the risk of developing diagnostically verified thyrotoxicosis at age before the given one. The curves of both sexes rise evenly with age showing a total life risk of about 5% for women and of about 1% for men.

The estimated cumulative risk figures are related to the age groups of the entire population of Funen region, an assessment can be obtained of the prevalence of current and previously diagnosed no longer present hyperthyroidism. The crude prevalence rate for women can then be assessed as 1.4% and for men as 0.3%.

DISCUSSION

In contrast to earlier studies the present investigation includes both inpatients and outpatients diag-

nosed by physicians outside the hospital. As the latter group constitutes only 11% of the total series we can conclude that new cases of thyrotoxicosis are in general admitted to hospital for establishment of the diagnosis and institution of treatment.

The validity of our data rests primarily on the fact that physicians in the region under study were aware of the significance of any thyrotoxic symptomatology. As it is impossible to provide evidence for this on the basis of the data from our study we have obtained the number of serum thyroxine tests performed in the study region during 1974. Of a total of 16 714 tests, 14 000 are assumed to relate to approximately 10 000 persons with suspected new thyrotoxicosis and this diagnosis was established in only 116 cases during 1974. Thus the common use of thyroid function tests seems to indicate that physicians are in fact aware of thyrotoxic symptomatology. On the other hand it must be presumed that no diagnosis would be made without laboratory confirmation and therefore we conclude that our series includes all newly diagnosed cases of thyrotoxicosis within the limits of time and territory set for the study.

In the present study, as in previous ones (6, 7, 8, 10), there is evidence of a distinct sex difference, the ratio between overall incidence rates in women and men being approximately 5:1 (Table I). This difference is repeated in all age groups although it appears to be less pronounced in the youngest and oldest patients.

Earlier epidemiological studies have shown an age peak for thyrotoxicosis in patients aged 0–10 years. Iversen (7) was the first to point out that this peak has tended to shift toward the higher age groups in the course of this century. Two recent Danish studies (8, 10) have shown that elderly patients now constitute a comparatively large proportion of newly diagnosed cases of thyrotoxicosis. In the former of these studies (8) the majority of patients were older than 60 years. Our investigation confirms that the highest incidence rate has now shifted to those 70 years of age or older.

This development is probably due to several factors. Firstly, doctors have become more familiar with atypical forms of the condition, especially in older patients. Secondly, diagnostic methods have improved considerably. A difference in the frequency of admission to hospital within the various age groups might be another factor, but this explanation is not supported by our results since only

Table III Incidence rates of thyrotoxicosis in various studies

Reference	Period	Country	Annual incidence rate per 100 000		
			♂	♀	♂ + ♀
Iversen (7)	1938-41	Denmark	5.7	45.6	24.6
Iversen (7)	1942-44	Denmark	24.4	148.3	81.5
Furszyfer et al. (6)	1935-67	USA	8.3	36.8	19.8
Thyrdleifsson (9)	1938-67	Iceland			1*
Thommesen et al. (10)	1966	Denmark	8	44	22
Present study	1972-74	Denmark	8.7	46.5	27.6

about 10% of all patients (representing all age groups) had not been admitted to hospital at the time of diagnosis. It cannot be ruled out, however, that the hospitalized patients in earlier studies may have been collected during periods when the admission frequency, particularly with regard to the elderly, was lower than now.

As shown in Table III, the overall incidence in our study is identical with that in previous studies. The higher occurrence observed in the elderly has not affected materially the size of the overall incidence rate, since the elderly represent only a small proportion of the general population. The present incidence rate in Denmark is similar to that before the Second World War. An epidemic of thyrotoxicosis in Denmark in 1942 and 1943 described by Iversen (7) appears to have been of a temporary nature without lasting effect on the incidence rate.

In the Minnesota study (6) a higher incidence of thyrotoxicosis was found among the city population than among the rural, yet the authors did not attach any great importance to this finding. We found a significantly higher incidence rate among women from areas with a low degree of urbanisation than in the Odense area (city area). This disparity can hardly be ascribed to any difference in medical practice, since the standard of medical service in Denmark is very homogeneous and does not differ between urban and rural areas.

The cumulative morbidity risk (Fig. 1) has been estimated on the assumption that all members of the study population throughout their lives and up to the conclusion of the survey had been exposed to the same age specific morbidity risks. Correspondingly, for the application of the graphs to future populations, it is a presupposition that these conditions are constant. A further prerequisite to estima-

tion of the prevalence is the assumption that thyrotoxicosis is not associated with an excess mortality.

Giving these premises, it can be concluded that in the Funen region, and presumably in the whole of Denmark, 1.4% of women and 0.3% of men have had thyrotoxicosis. This is not far from the prevalence reported recently from England. Tunbridge et al. (11) found a prevalence of 1.9% females and 0.16% in males.

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The Effect of Beta-Blockade on Glucose Tolerance and Insulin Release in Adult Diabetes

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ABSTRACT Blood glucose and plasma insulin levels were studied in ten adult diabetics treated in a cross over fashion for at least three weeks with alprenolol, a cardioselective β blocker, or with metoprolol, a non-selective β blocker. Dietary intake was controlled during the study which comprised both oral and intravenous glucose tolerance tests. Mean fasting glucose levels were significantly higher on alprenolol than on metoprolol. The increase in fasting glucose was particularly pronounced in two patients. In these subjects the glucose tolerance failed both an intravenous and an oral glucose load was used when treatment was switched from metoprolol to alprenolol. Lower plasma insulin levels in response to glucose were also found in these patients on alprenolol than on metoprolol. The mean insulin release for all ten patients did not differ significantly between the two treatment periods. These data show that treatment with a non-selective β blocker can in some patients cause a considerable deterioration of glucose tolerance, presumably due to inhibition of insulin release.

Keywords: alprenolol metoprolol
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Diabetics run an increased risk of developing various cardiovascular disorders such as ischemic heart disease (IHD) and hypertension. Consequently treatment with different agents such as β adrenergic receptor blocking agents or diuretics may be considered. It seems quite clear that diuretics may impair glucose tolerance in some non-diabetic subjects (2, 13). Only few studies on the effect of β blockade on glucose tolerance have appeared. In non-diabetic subjects β blockers influence glucose tolerance only to a small extent or not at all (17). However, there are reports of clear reductions in glucose tolerance in some patients (5,

18). It appears that there may be differences in this respect between a cardioselective β_1 blocker and a non-selective β blocker (18). The reason for this may be the presence of β receptors in human pancreas (19).

It is particularly important, however, to evaluate the influence of β blockade on blood glucose control in diabetics. Wright et al. (20) reported recently that β blockers may to a small extent raise the blood glucose levels during the day in diabetics. There was no significant difference in this respect between a β_1 selective (metoprolol) and a non-selective (propranolol) drug although the mean blood glucose levels were lower on metoprolol at all sampling times. In order to evaluate the effect of drugs on blood glucose levels it is necessary to control the dietary intake. Such a control does not appear to have been done in that study.

We have investigated the tolerance to an intravenous and an oral glucose load in ten adult diabetics while treated in a cross over fashion with metoprolol or a non-selective β blocker alprenolol. The dietary intake was controlled during three days prior to the glucose tolerance tests.

PATIENTS AND METHODS

Ten adult diabetics, nine of whom required sulfonylurea treatment, were studied. Six patients were treated with alprenolol 200 mg b.i.d. and four with metoprolol 100 mg b.i.d. due to IHD. Their diabetes was considered well controlled and they had been seen regularly on an outpatient basis. The clinical characteristics of the patients are shown in Table I. The alprenolol and metoprolol doses given are considered to give equipotent blockade of the β receptors (2, 11).

The patients were given an isocaloric formula diet containing 16% protein, 45% carbohydrate and 39% fat in

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Table I Clinical characteristics of the diabetic studied

Pat no	Age (y)	Sex	B wt (kg)	Known duration of IHD (y)	Duration of diabetes (y)	Therapies besides β blockers
1	50	♂	85	7	1	Gl benclamide 7.5 mg \times 1
2	56	♂	90	4	3	Chlorpropam de 250 mg \times 1
3	65	♂	75	13	6	Chlorpropam de 250 mg \times 1
4	65	♂	73	7	15	Chlorpropam de 250 mg \times 1
5	61	♂	106	9	6	Chlorpropam de 250 mg \times 1 met / 4
6	77	♂	73	6	6	Gl benclamide 5 mg \times 1
7	60	♂	84	10	3	Chlorpropam de 250 mg \times 1
8	48	♂	122	2	1	Det
9	58	♀	86	11	7	Chlorpropam de 37.5 mg \times 1 met / 0.5 g \times 7
10	54	♂	96	4	1	Tolbutam de 0.5 g \times 7

preweighed individual portions to be taken three times daily at mealtime for three days prior to the glucose tolerance tests. The patients came to the laboratory in the morning after an overnight fast. They had taken the last alprenolol or metoprolol dose one hour before the glucose tolerance tests. Venous blood samples were drawn for the analysis of blood glucose using a glucose oxidase technique and plasma insulin levels (Phadebas Pharmacia Uppsala, Sweden). I.v. glucose (0.5 g/kg B.Wt.) was then injected rapidly and venous blood samples were drawn at the indicated times. On the next day the patients came again to the laboratory and an oral glucose tolerance test (100 g glucose dissolved in 700 ml water) was performed under the same conditions.

After the tolerance tests the patients were switched to the other β blocker (i.e. alprenolol instead of metoprolol and vice versa) and the study was repeated in an analogous way after a treatment period of at least three weeks. Any other concomitant medication was continued unchanged throughout the study.

RESULTS

I.v. glucose tolerance

Table II shows the individual fasting blood glucose levels before the i.v. and oral glucose tests. Most patients (7 and 6 of 10 respectively) had higher glucose levels on alprenolol than on metoprolol although the values did not generally differ markedly. Patients 9 and 10 however had considerably higher fasting levels on alprenolol (Table II).

There was no significant difference in the peak glucose levels reached during the i.v. tolerance tests although all values were somewhat higher on alprenolol (Fig. 1). The mean insulin level during the glucose load are also depicted in Figure 1. It is clear that there were great inter-

Table II. Fasting blood glucose (mM) in 10 diabetic subjects after at least 3 weeks treatment with alprenolol or metoprolol

Pat no	Before i.v. test		Before oral load	
	Alprenolol	Metoprolol	Alprenolol	Metoprolol
1	7.2	7.0	6.8	5.6
2	6.9	5.0	6.3	3.5
3	10.5	11.1	10.2	12.0
4	7.2	7.5	8.5	7.1
5	5.7	5.4	5.8	5.8
6	6.1	6.0	7.3	7.8
7	3.8	4.3	3.4	3.6
8	8.9	8.0	9.1	8.9
9	11.0	8.1	11.0	7.3
10	10.8	6.9	10.8	6.6
Mean \pm S.D.	7.8 \pm 2.4	6.9 \pm 1.9	7.9 \pm 2.4	6.8 \pm 2.5
	$p < 0.05$		$0.05 < p < 0.1$	

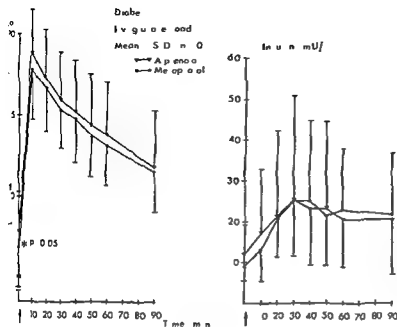


Fig 1 Glucose and insulin levels in response to an i.v. glucose load (0.5 g/kg b.wt.) in 10 adult diabetics treated with alprenolol or metoprolol for at least three weeks

ences and no significant change was found in the two drugs.

Glucose tolerance of patients 9 and 10 whose blood glucose levels were considerably reduced by alprenolol (Table II) was analysed separately. As shown in Fig 2 the oral glucose tolerance was considerably reduced by alprenolol and was associated with a reduced insulin release during the load.

Oral glucose tolerance

Essentially similar results were obtained as with the i.v. tolerance test. The fasting blood glucose levels were generally higher on alprenolol (Table II). The insulin response to glucose was not significantly different but again the insulin levels were lower on alprenolol in patients 9 and 10 and this was associated with higher blood glucose levels during the tolerance test (Fig 3).

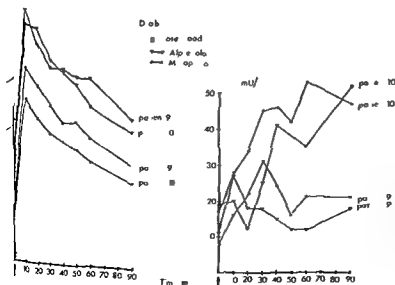


Fig 2 Glucose and insulin levels in response to an i.v. glucose load in patients 9 and 10 treated with alprenolol or metoprolol for at least three weeks

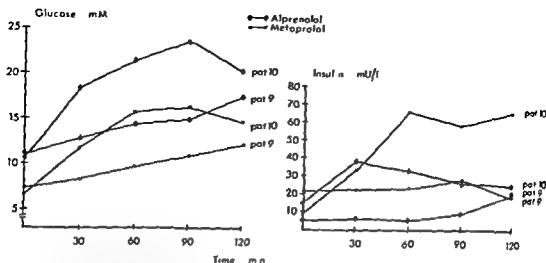


Fig 3 Glucose and insulin levels in response to an oral glucose load (100 g) in patients 9 and 10 treated with alprenolol or metoprolol for at least three weeks

DISCUSSION

In a recent study it was shown that stimulation of β_1 receptors leads to insulin release in man (19). However, the importance of the β receptor for glucose-stimulated insulin release is not clear although an association is suggested by the observation that glucose-stimulated insulin release is greatly augmented by simultaneous β receptor stimulation (7). Also, significant changes in insulin release after β blockade in non-diabetics were not noted in some studies (8, 9, 17) but were found in some others (5, 18). The reason for this may be that adrenergic mediated insulin release is of quantitative importance in some patients. Cerasi et al (4) for instance found a clear reduction in insulin release in some patients while their other patients were unaffected. The consensus that insulin release may be reduced by non-selective β blockade in some patients is also exemplified by the fact that patients with insulinoma can be successfully treated with propranolol (3). Other studies have shown that sulfonylurea-stimulated insulin release may also be reduced by β -blockade (14, 15).

The present investigation was designed to study the influence of β -blockade on glucose control in diabetics. In order to eliminate effects of possible changes in the diet, caloric intake was controlled for three days prior to the study. The data show that the non-selective β -blocker alprenolol raised the fasting glucose levels slightly in most patients but

this effect was pronounced in two of the patients. The deterioration in glucose tolerance in these patients seems to be due to a low insulin release. In these patients then β mediated insulin release appears to be of quantitative importance. Neither from a clinical point of view nor mode of medication were there any apparent differences between these two patients and the others. It is of course also feasible that blockade of β_1 receptors leads to an unopposed stimulation of catecholamines thereby inhibiting insulin release (16). Further studies are required to elucidate this point.

Irrespective of the mechanisms involved it appears that a non-selective β blocker in the administered clinical doses influences glucose tolerance in adult diabetics to a greater extent than a cardioselective β_1 blocker.

Since cardioselective β_1 blockers not only decrease glucose tolerance but also are a potentially hazardous complication of the disease state, namely recovery from hypoglycaemia, it appears that these agents are preferable if a β blocker is required in diabetics.

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Castle's Test (with Vitamin B₁₂ and Normal Gastric Juice) in the Ileum in Patients with Genuine and Patients with Tapeworm Pernicious Anaemia

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ABSTRACT A mixture of vitamin B₁₂ and normal gastric juice, instilled through an intestinal tube into the ileum, produces haematological remission in patients with tapeworm pernicious anaemia. When a mixture is administered by mouth, this effect does not occur. This observation constitutes evidence in favour of the view that *Diphyllobothrium latum* resides in the proximal portion (jejunum) of the intestine, absorbs the vitamin B₁₂ contained in the gastric juice, thus preventing vitamin bound to the intrinsic factor of the gastric juice from reaching the receptors in the distal portions of the small intestine of the host. In genuine pernicious anaemia remission results from the administration of vitamin B₁₂ + gastric juice by mouth and into the ileum.

Key words: *Diphyllobothrium latum*, pernicious anaemia, B₁₂ absorption.

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Monograph published in 1977 (4) (below referred to as II monograph) the kinetics of vitamin B₁₂ in tapeworm pernicious anaemia (PA) and the genesis of this disease are discussed. The graph represents a recapitulation of investigation on this problem complex, which for the part of 10 years of workers were concluded about 1960. The present paper is an addendum to the mono-

graphing 1949-54 we performed Castle's test with normal human gastric juice (GJ) introduced into the ileum by intestinal intubation in 4 patients of genuine PA and 13 of tapeworm PA. The tests were discontinued when radioactive B₁₂ became available and it was considered more important to study *D. latum* infection by the isotope technique. At about the same time we were more interested in the microbiological determination of B₁₂ in the blood and in the parasite. The

reports of the above mentioned experiments were put aside and finally fell into oblivion. They have recently been found again and the results seem valuable enough for publication.

METHODS

One of us has described intestinal intubation of *D. latum* carriers for the demonstration of tapeworm ova in the intestinal contents (2). The distance from the mouth to the level where tapeworm ova were found was 140-135 cm (mean 170) in 11 cases of *D. latum* infection without anaemia, 95-135 cm (mean 115) in 11 cases of manifest tapeworm PA. The distance from the mouth to the duodenojejunal flexure was estimated at 70-80 cm, to the junction between the jejunum and the ileum at 140-160 cm and to the ileo caecal valve at 250-300 cm.

In the experiments to be described below the same principles as in oral administration of Castle's test were applied. After intubation to a level of 145-200 cm a mixture of 5 µg B₁₂ and 100 ml depepsinized GJ daily was instilled through the tube in 8-day periods. Parallel tests were performed without GJ, with 5 µg of B₁₂ diluted in 100 ml of water. The position of the tube was radiologically checked. Other measures appear in the diagrams.

RESULTS

Genuine PA

A good haematological response was obtained in all 4 cases in this group. A typical course is shown in Fig. 1. At a level of 145 cm B₁₂ + GJ gave remission and a stronger reticulocytosis than the same mixture administered by mouth during a subsequent test period. An identical result was obtained at 165 cm when 5 ml of an injectable liver preparation (Heptomin®) was used instead of pure B₁₂ (Fig. 2).

Abbreviations: B₁₂ = vitamin B₁₂; PA = pernicious anaemia; GJ = gastric juice; IF = intrinsic factor.

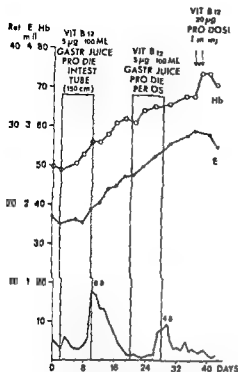


Fig. 1 Genuine PA Woman 54 years E = erythrocytes (mill/mm^3) 100% Hb corresponds to about 14 g/ml Ret = reticulocytes (% of E)

shows an experiment in which 100 ml GJ alone was instilled (at 200 cm) during the initial period. Definite reticulocytosis and hematological remission ensued. However the effect was stronger during the second trial period when GJ was given in combination with B_{12} . The fourth patient in this group received 100 ml GJ daily by mouth during the first trial period with no result. B_{12} + GJ administered after intubation (200 cm) produced a good remission (maximum reticulocytosis 13.3%).

Tapeworm PA

Rapid remission resulted in 4 cases after instillation of B_{12} + GJ into the ileum (at 150 cm). Two experiments are reproduced in Figs. 3 and 4.

The effect of B_{12} alone administered first into the ileum and subsequently by mouth was studied in 9 cases. The results were as follows:

A) Instillation into the ileum caused reticulocytosis and moderate or no improvement of the blood values (Hb, E). (a) Administration by mouth (one case, Fig. 5) resulted in marked remission. (b) Administration by mouth (5 cases) produced no remission. One case is illustrated in Fig. 6. In another case (Fig. 7) a weak reticulocytosis

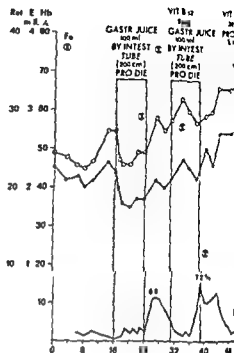


Fig. 2 Genuine PA Man 72 years

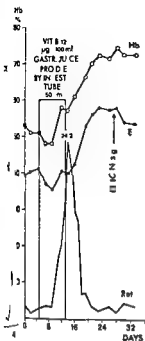
without remission was observed. B mouth had no effect either. Two blood tests had a slight influence on the curves.

B) Instillation into the ileum failed to produce reticulocytosis. The blood values dropped. Administration by mouth was followed by reticulocytosis (7.2%) and moderate remission (case (b)). Administration by mouth resulted in neither reticulocytosis nor any improvement of blood values (2 cases). In one case Bend's commercial preparation of B_{12} (and pig iron factor (IF) then commonly used) given before expulsion of the worm produced no remission, as was usually the case in both PA and genuine PA.

DISCUSSION

Genuine PA

In the early 50s it was not yet clearly established where the absorption of B_{12} takes place. In the above mentioned study of 4 cases we were, to our knowledge, the first to show that B_{12} is absorbed in the ileum in the presence of GJ. These results were shortly mentioned at the Europäisches Symposium über Vitamin B₁₂ und Striktor Factor in May 1956 (1). Later it



Tapeworm PA Woman 45 years I v insulin test 79 total ac d ty 73

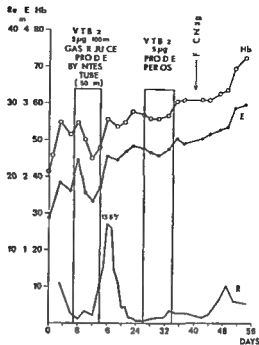


Fig 4 Tapeworm PA Woman 38 years Insulin test not made

ed
shed that the II absorption is princ pally
led by receptors located in the distal port on
leum The problem is discussed in the D
graph (4) p 145

In one case GI w thout any B₂ adm n stered
at a level of 700 cm produced a submax mal
haematolog cal response (Fig 7) A sim lar effect
was never obta ned w th GI alone by mouth Obv

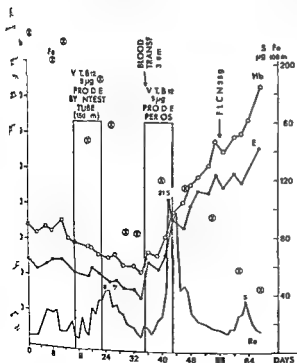


Fig 5 Tapeworm PA Woman 40 years I v insulin test free HCl 27 total ac d ty 45

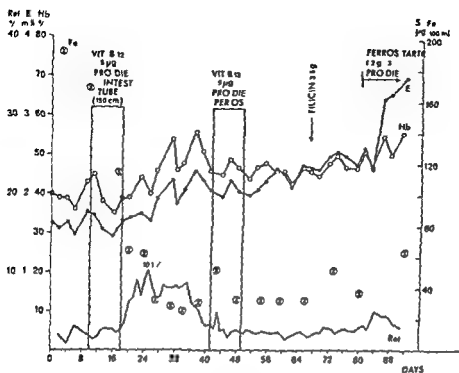


Fig. 6. Tapeworm Woman 61 years hydatid

ously the remission in this case was due to the presence of small amounts of B_{12} in the ileum either derived from the food or synthesized in the distal portion of the small intestine.

Tapeworm PA

Castle's classical test administered by mouth fails to produce remission in tapeworm PA. However in those 4 cases in whom we instilled B_{12} + GJ into the ileum reticulocytosis and blood remission occurred. For the sake of control oral administration of B_{12} ought to have preceded the instillation into the ileum in at least one experiment. On the other hand the failure of Castle's classical test in tapeworm PA may be considered an established fact on a basis of a very large number of previous experiments (3, 5) (Fig. 7).

In contrast to genuine PA the endogenous production of IF has not ceased in tapeworm PA (D monograph (4) pp. 135-170-173). The effect of B_{12} without any addition of GJ has therefore been tested in tapeworm PA. Orally administered B_{12} alone gave either no haematological response or a remarkably good remission (5). These results are discussed in the D monograph p. 146.

The instillation of B_{12} alone into the ileum in tapeworm PA gave variable results. In some cases unused IF seems to have been present in the ileum in other cases not. When the IF- B_{12} complex is

exposed to the action of D latum in the intestine and the B_{12} is absorbed by the worm component is obviously split off and reabsorbed. Even under normal conditions the surplus

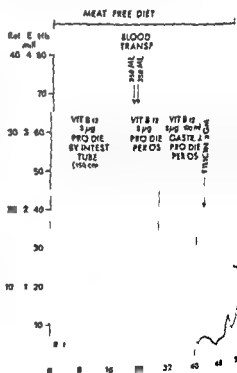


Fig. 7. Tapeworm PA Woman 76 years A 12

ably degraded during its passage through the final canal (D monograph p 161) though in cases small amounts apparently get as far as cæcum

According to our theory of the pathogenesis of worm PA the disease originates from a combination between the host and the parasite for the contained in the food. Among the circumstances red for the development of tapeworm PA (monograph pp 175-178) the situation of the parasite in the small intestine is of decisive importance.

The higher up in the jejunum the parasite has become attached the greater the risk that it will be for itself the B_{12} that the host organism is in need of. The evidence in favour of this theory is

In the D monograph (p 163) a test program outlined which would further elucidate the ques-

tion the ileum would lend further support to the theory set forth above

However the most essential points of the problem have been clarified by the experiments reported above performed by an earlier more laborious technique. The most important issue is the finding that remission results in tapeworm PA if B_{12} + GJ are administered into the ileum and tapeworm situated higher up in the intestine is thus prevented from interfering. These phenomena could no doubt be elucidated in greater detail utilizing the isotope technique but it may be questioned whether the gain would be worth the trouble considering the insight already acquired.

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The Relation between the Levels of HDL Cholesterol and the Capacity for Removal of Triglycerides

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ABSTRACT In twenty-two men with normal is clinically by exercise ECG and laboratory performed an iv fat tolerance test : means of LCAT activity and the activities of post triglyceride lipases ; and estimated serum HDL cholesterol was positively correlated to plasma lipase ($r = 0.40$ $p < 0.05$) and to the fractional removal rate of exogenous fat ($r = 0.59$) and negatively to the fasting levels of triglycerides ($r = -0.43$ $p < 0.01$). LCAT was neither correlated to HDL cholesterol nor to the fat removal rate. Our results confirm that a high triglyceride removal capacity is closely correlated to a high level of HDL. The association between the catabolism of triglyceride rich lipoproteins and HDL was thus substantiated.

High density lipoprotein lipase
Fractional removal capacity
Acta Med Scand 708 199 1980

Studies have suggested that high concentration of high density lipoprotein (HDL) may have a protective role against coronary atherosclerosis (7).

The mechanism involved is unknown, but it has been postulated that HDL/lecithin cholesterol acyltransferase (LCAT) may remove cholesterol from the arterial wall (11) or that HDL may prevent the oxidation of cholesterol rich lipoproteins (6).

Lipoproteins are synthesized and secreted by the liver (15) and by the intestine (14). A close negative relation exists between very low density lipoprotein (VLDL) concentration and HDL exists. This is reflected by a negative correlation between plasma triglyceride concentration and HDL both in normal subjects and in patients with hyperlipoproteinemia (18). A positive correlation between HDL cholesterol and plasma lipoprotein lipase has recently been reported by Nikkila et al (25). Eisenberg (8) has focused upon lipoprotein lipase as the link be-

tween VLDL and HDL. A positive correlation between HDL and the removal rate of intravenously administered fat has recently been reported (17).

In order to elucidate further the relationship between triglyceride catabolism and HDL concentration in plasma, we studied lipoprotein lipase activity in postheparin plasma and the fractional removal rate of an exogenous lipid in relation to HDL cholesterol in healthy male subjects.

SUBJECTS AND METHODS

In 1977-78 7014 apparently healthy male employees (aged 40-59 years) from five major companies/governmental agencies in Oslo, Norway, participated in a cardiovascular survey (9). In 1966-63 of the 1837 subjects defined as normals in that survey were reexamined clinically by exercise ECG and serum lipid analyses. Two of the 1837 (both with low HDL cholesterol) had suffered a myocardial infarction since the primary survey and were excluded. None of the others had symptoms or signs of coronary heart disease (CHD). Twenty-two of the remaining 61 normals were selected for the present study (performed in 1977-78) representing the 11 highest group I (mean age 53 years) and 11 lowest (group II, mean age 49 years) levels of HDL cholesterol in 1976. No differences in habitual physical activity, alcohol consumption or smoking habits was found between the two groups. Fasting blood glucose and insulin were within the reference range in all subjects studied. None had hypertension and none was on lipid lowering drugs or drugs. None of the subjects

The present data have been presented at the Åkarp Meeting on HDL in Aug. 1978 (ref. 30).

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Abbreviations: HDL = high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein; LCAT = lecithin cholesterol acyltransferase; LCAT SN = LCAT according to Stokke & Norum; LCAT GW = LCAT according to Glomset & Wright; CHD = coronary heart disease.

Table 1 Plasma lipids, Intralipid removal and activities of lipases and LCAT in Group I and II
Group I = 11 men with mean HDL cholesterol level of 1.77 mmol/l (S.D. 0.23); group II = 11 men with mean HDL cholesterol level of 1.09 mmol/l (S.D. 0.19)

	Group I		Group II	
	Mean	S.D.	Mean	S.D.
Total cholesterol (mmol/l)	5.9	1.1	5.0	0.8
Triglycerides (mmol/l)	0.7	0.3	1.4	0.6
Intralipid removal (%/min)	3.4	1.2	2.1	0.8*
Lipoprotein lipase (μ mol FFA/ml plasma/h)	14.4	4.3	10.8	3.1*
Hepatic lipase	21.0	6.6	25.8	7.8
LCAT S.N. (μ mol/l/h)	53	10	64	19
LCAT G.W. (μ mol/l/h)	111	22	119	25

* $p < 0.05$ ** $p < 0.01$ for lack of significance of difference between the groups calculated by Student's *t* test

had symptoms of CHD but exercise ECG showed ischaemia in two group II subjects.

Blood sampling and investigations related to triglyceride catabolism were performed in the morning after 12–14 hours fast and after 15 min rest with the subjects in prone position. The subjects were on their ordinary diet. They were told not to drink alcohol on the day before the examination. Since the previous survey showed that alcohol consumption took place mainly during the week ends (10) the investigations were performed in the middle of the week. Heparin was injected for subsequent analyses of plasma lipoprotein lipase activity on the day after measurement of the removal rate of an exogenous lipid load.

Triglyceride removal capacity was measured as described by Nikkila et al. (24) by i.v. infusion for 10 min of 20% Intralipid[®] (Vitrum Sweden) 0.1 g/kg b.wt. The % of Intralipid disappearance was calculated from the linear slope of the curve obtained from serum triglyceride measurements at 10, 15, 20, 25 and 30 min after the end of the infusion and was plotted against time in a semi-logarithmic plot.

Lipase activity was assayed in plasma samples drawn 15 min after i.v. administration of heparin 100 U/kg b.wt. (29). Lipoprotein lipase and hepatic lipase activity were determined by the immunoprecipitation method described by Huttunen et al. (16). After preincubation with specific antiserum against hepatic lipase the lipoprotein lipase activity in an artificial substrate of triolein was tested as the remaining triglyceride lipase activity at 0.1 M NaCl in the presence of serum as cofactor source.

Total cholesterol and triglycerides were measured by methods adapted for AutoAnalyzer (10). HDL cholesterol was estimated by an enzymatic method (79) after precipitation of VLDL and LDL with heparin and methyl cellosolve. Analyses of free cholesterol were performed by chromatography (3). LCAT activity was measured according to Stokke and Norum (31). LCAT Glomset and Wright (13). LCAT G.W. Heur plasma from one healthy subject served as a substrate source for LCAT G.W. analyses.

Spearman's rank correlation test and Student's *t* test were used for statistical analyses.

RESULTS

The level of HDL cholesterol present in subjects examined was significantly correlated with the level found at examination of stored sera drawn 3–6 years ago ($r = 0.61$, $p < 0.01$) and sera drawn and assayed one year ago ($r = 0.83$, $p < 0.01$).

Table 1 shows that total cholesterol and triglycerides, the intralipid removal rate and lipase activities were significantly different between Group I and II. Only one subject had a serum triglyceride level higher than 1.7 mmol/l (2.3 mmol/l). All total cholesterol levels were within the normal range in all (i.e. < 7.5 mmol/l). The difference

Table 2 Correlation matrices between triglycerides, Intralipid removal, lipase and LCAT activities

	Triglycerides	Lipoprotein lipase	Hepatic lipase	Intralipid removal	LCAT G.W.
Lipoprotein lipase	-0.96				
Hepatic lipase	0.24	0.17			
Intralipid removal	-0.71*	0.28	-0.48		
LCAT G.W.	0.42	-0.37	0.02	-0.25	
LCAT S.N.	0.81	-0.27	0.15	-0.17	0.44

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ calculated by Spearman's rank correlation test

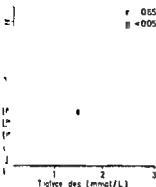


Fig 1 Comparison of plasma triglycerides and HDL cholesterol level

level in the two groups caused almost the same difference in total cholesterol levels. The triglyceride concentration was inversely correlated to lipoprotein lipase activity and to the removal rate of an exogenous fat load but positively related to LCAT activity (Table II). There was no significant relationship between triglycerides and hepatic lipase activity. The fat removal rate was negatively correlated to hepatic lipase activity but not to lipoprotein lipase activity (Table II). The concentration of HDL cholesterol was negatively correlated to triglycerides (Fig. 1) but positively to the removal rate of Intralipid® (Fig. 2) and activity of lipoprotein lipase (Fig. 3). There was no significant relationship between HDL cholesterol and hepatic lipase activity ($r = -0.33$) or LCAT activity ($r = -0.17$ for LCAT S-N, $r = 0.07$ for LCAT G-W).

DISCUSSION

Epidemiological studies have suggested that HDL cholesterol is of significance for the pathogenesis of atherosclerosis (7, 18, 21). The regulation of formation and catabolism of these lipoproteins is, however, only incompletely understood (21). The present investigation of essentially normolipidemic middle-aged men has confirmed a close relationship between the concentration of HDL cholesterol and activity of lipoprotein lipase (21, 25) and also the removal capacity of exogenous lipids (17). Our findings are compatible with the formation of HDL in plasma through catabolism of triglyceride-rich lipoproteins. The results agree well with recent reports by Nikkila et al. (21, 25) on the relation be-

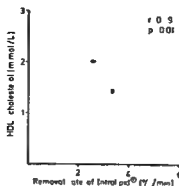


Fig 2 Removal rate of Intralipid® in relation to HDL cholesterol level

tween HDL and adipose tissue lipoprotein lipase and may at least partly explain the inverse relation between the concentration of plasma triglycerides and HDL which has been found also in other studies (18, 22). The importance of catabolism of VLDL (or chylomicron (32)) by lipoprotein lipase as a regulating factor for HDL concentration is also supported by *in vitro* studies by Nikkila (23) and Eisenberg (8) and by reports on rather low HDL concentration in patients with type I hyperlipoproteinemia or familial LCAT deficiency (lipoprotein lipase activity is low also in the latter condition (4)).

The studies by Glomset et al. (12) in patients with familial LCAT deficiency have demonstrated a key role of LCAT in lipoprotein metabolism. The preferred substrate for this enzyme is HDL (11). In the present investigation no significant relation between LCAT activity and HDL cholesterol was

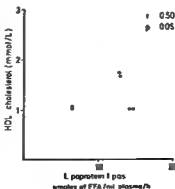


Fig 3 Comparison of lipoprotein lipase activity and HDL cholesterol level

found. However, this is not necessarily in contrast with the suggestion by Glomset (11) that LCAT is important for removal of cholesterol from cells. Important functions of this enzyme in lipoprotein catabolism and cholesterol transport may not be reflected by the total *in vitro* capacity for plasma cholesterol esterification measured by the two methods used in this study. The strong positive correlation between plasma triglycerides and LCAT—also observed previously (1)—might be explained by a concomitant secretion of LCAT and VLDL by the liver, as suggested by Nordby (26).

Men who are physically active during their leisure time may have a reduced risk of coronary atherosclerosis (20, 33), increased HDL levels, lipoprotein lipase activity (22) and removal rate of exogenous lipids (2, 17). All these features are in contrast to uncontrolled insulin-dependent diabetes (22, 24). On this background, our results support the concept that a high triglyceride removal capacity is another marker for individuals at low risk for CHD, even though VLDL may not be a separate risk factor (7).

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HDL-Increasing Effect of Cyclofenil

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TRACT Nineteen patients with low levels of as measured by APO AI have been treated with cyclofenil (a non steroid stilboestrol isomer with very oestrogenic effects) as the only form of therapy. Therapy elicited a highly significant rise of about 100% in HDL levels. A slight increase in triglycerides occurred in 6 patients but was reversible on terminating therapy.

Keywords: lipoproteins, HDL, oestrogens, cyclofenil, atherosclerosis, coronary disease, preventive medicine.
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In recent years a growing body of research has shown that there is a very strong negative correlation between the plasma HDL (high density lipoprotein) level and the probability of developing a heart attack (1, 2, 3, 4, 5, 6, 7, 8). The evidence comes both from epidemiological studies in which decreased concentrations of plasma HDL have been linked with increased extent of coronary artery occlusion, peripheral artery disease (3) and risk of heart attack (9) and from *in vitro* studies in which HDL has been shown to influence uptake and release of cholesterol (4, 10). The relationship seems to hold both for HDL cholesterol and HDL lipoproteins AI (APO AI) (3, 6, 9) and AII (3). Other factors such as oestrogens (11), exercise and alcohol (10) influence the HDL level. It is of great interest to find ways of elevating the plasma HDL level in order to study whether this is able to decrease or eliminate the increased risk for arterial disease and heart attack. Low HDL seems to constitute

a weakly oestrogenic compound with anti-oestrogenic properties. Its oestrogenic effect is 1/1000 of native oestrogen (8). Therefore it can be administered to men without oestrogenic side-effects. It has been used with good results in the treatment of scleroderma (PSS) (2) as well as in gynaecological practice.

PATIENTS

From a general preventive screening for risk factors undertaken at the Department of Preventive Medicine, Medical Clinic of Malmö General Hospital, we selected 19 consecutive patients (aged 47-53 years) whose levels of APO AI placed them in the lowest five per cent and were thus below 90. These patients were screened with three separate analyses for APO-AI and their informed consent was obtained prior to participation in the study. The 0-level was calculated and the patients were controlled bimonthly with the tests mentioned below. The patients were instructed not to change their therapy, diet, exercise or daily living conditions during the study. The initial dose of cyclofenil was 200 mg \times 3.

METHODS

Blood samples were drawn in the morning in the fasting state. Serum was stored at +4°C until analysed within 48 hours. Antiserum to human HDL isolated by ultracentrifugation was raised in rabbits and was found by crossed immunoelectrophoresis (7) to react only with APO AI.

Serum cholesterol (15) and triglycerides (18) were determined enzymatically. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activity were determined with reagents from Boehringer Mannheim using a Vitatron Akos reaction rate analyser. Gamma-glutamyl transferase was determined with glutamyl p-nitroanilide as substrate (14).

APO AI was determined by electroimmunoassay with frozen pooled human serum as standard as previously described (5). HDL cholesterol was determined after precipitation of low density lipoprotein (LDL) and very low density lipoprotein with dextran sulphate $MgCl_2$ (5).

CYCLOFENIL

In order to eliminate the increased risk factor for development of heart attacks that lies in a low concentration of HDL, we have used cyclofenil. Cyclofenil is a synthetic steroid isomer of diethylstilboestrol. It is a very

Abbreviations: HDL = high density lipoprotein, LDL = low density lipoprotein, APO AI = apolipoprotein AI, ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase.

Table I Clinical data and effects

Pat no	Sex	Age (y)	APO AI (nmol/l)				Other previous disease	Side effects	Change after therapy
			Initial	2 mo	4 mo	6 mo			
1	♂	48	78	90	95	95	None	None	
2	♂	47	83	110	95	115	Eczema	Slight reversible rise in ALAT	Diminished after 4 mo
3	♂	48	86	90	105	100	Hypertension obesity	Thinks more clear	
4	♂	57	117	175	115	115	None	None	
5	♂	53	105	170	110	110	Angina pectoris	None	
6	♂	48	91	125	130	115	Ulcer duodeni	Slight reversible rise in ALAT	Diminished after 4 mo
7	7♂	47	87	90	100		Ulcer duodeni	Slight reversible rise in ALAT	Diminished after 4 mo
8	♂	48	88	105	170	105		epigastric pain	Diminished after 4 mo
9	♂	47	77	80	85		Hypertension urinary infection	Slight reversible rise in ALAT	Diminished after 4 mo
10	♂	49	85	100	100		Gastritis	heavy alcohol intake	
11	♂	48	95	100			None	Slight reversible rise in ALAT	Diminished after 4 mo
12	♂	49	85	105	115	95	Ulcer ventriculi	None	Diminished after 4 mo non-recurred
13	♂	48	90	110			Renal calculus	None	
14	♂	48	93	95			Lumbago	None	
15	♂	47	88	105			Gastritis amputated limb	Slight symptom of gastritis	Diminished after 1 mo
16	♂	47	90	105	135		None	None	
17	♂	48	89	110					
18	♂	48	85	100			Hypercholesterolaemia takes clofibrate	Urinary	Improved
19	♀	49	95				Eczema menopause problems	Eczema disappeared vaginal bleeding	Improved

RESULTS

Table I gives the individual results and complications during the study.

As indicated in Table II APO AI levels showed a highly significant rise of about 15% after two and four months of therapy ($p < 0.000$ and 0.013 respectively). There was also a significant rise in HDL cholesterol and probably corresponding to this a tendency to a rising cholesterol level. LDL cholesterol calculated as total cholesterol minus (HDL cholesterol plus triglycerides/2.8) displayed a non significant tendency to increase.

Complications

There was a tendency to slightly lower blood pressure levels during treatment. The transaminases were slightly and transiently elevated in 6 patients. It is well known from the PSS treatment studies that a slight increase in ALAT is not quite uncommon on rather high doses of cyclofenil (600 mg). It was

reversed when the dose was reduced to the initial level (300 mg). In the non-accidental with daily doses of 700 mg, such increase is uncommon. As shown in Table II all 6 with a transaminase rise continued on the dose without further complications and at the higher APO AI level. Vaginal bleeding in patient 19 seems to be postmenopausal as it is affected of termination of therapy.

DISCUSSION

If one accepts the APO AI level or HDL cholesterol as an indicator of the HDL level, it is obvious that a rise in HDL levels can be obtained by treatment with cyclofenil. Measured as APO AI the increase was about 15%. Measured as HDL cholesterol the increase was less, which suggests an abnormal position of the HDL. A lower dose than the daily could presumably be used as the best

Table 1. Variables before and after treatment

	Time (mo)	No	0-level	After	P (P value probably)
AI (ref 5)	0-2	17	91.0	104.4	0.00
	0-4	10	97.6	108.7	0.13
cholesterol (mmol/l)	0-2	17	0.80	0.86	0.14
	0-4	11	0.79	0.84	0.13
triglycerol (mmol/l)	0-2	18	5.0	5.36	0.53
	0-4	17	5.1	5.5	0.51
glucose (mmol/l)	0-2	18	1.6	1.8	0.13
	0-4	17	1.47	1.7	0.31
T (μkat/l)	0-2	18	0.35	0.54	0.19
	0-4	17	0.36	0.67	0.86
T (μkat/l)	0-2	18	0.36	0.75	0.90
	0-4	17	0.39	1.03	0.15
diastolic BP (mmHg)	0-2	16	85.6	87.8	0.70
	0-4	11	86.8	84.5	0.13
cholesterol (mmol/l)	0-2	17	3.51	3.71	0.41
	0-4	11	3.66	3.89	0.41

was maintained even after the dose had been reduced in the 6 patients. Probably 300 mg daily will give the desired result and avoid reversible side effects on the transaminases. In the short period of observation one can only demonstrate that cyclofenil induces a highly significant rise in HDL levels. Whether this increase is sufficient to confer a reduced risk of heart attacks is not known for long term prevention studies.

ACKNOWLEDGEMENT

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Urinary Zinc Excretion during Treatment with Different Diuretics

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OBJECT Urinary zinc excretion was studied in a double-blind trial in 9 patients during treatment with bendroflumethiazide, chlorthalidone and hydrochlorothiazide and in another 9 patients during treatment with bumetanide, furosemide and triamterene.

During treatment with the thiazides, the zinc excretion rose by 30% and the total amount of zinc excretion increased by 60%. In contrast during treatment with the loop-diuretics, urine zinc concentration diminished and the total amount of zinc excretion increased much less than during therapy with the thiazides. With respect to the importance of zinc as an essential element in human metabolism and the effect of diuretic treatment, the observed increase in urinary losses of zinc deserve further attention.

During penicillamine therapy one patient developed a skin disorder resembling parakeratosis, a well-known zinc deficiency disorder in swine (10). Raised urinary zinc excretion has also been observed during treatment with chlorthalidone (19, 20) and hydrochlorothiazide (8).

The aim of the present study was to investigate the urinary zinc excretion during treatment with six diuretic drugs: bendroflumethiazide, bumetanide, chlorthalidone, furosemide, hydrochlorothiazide and triamterene.

PATIENTS AND METHODS

Eighteen patients with untreated arterial hypertension, 10 men and 8 women, ranging in age from 31 to 75 years, were included in the study. Some clinical data on the patients are presented in Table I. Their diastolic BP at the first visit to the hospital varied between 105 and 130 mmHg. Patients 1 and 2 had enlargement of the heart. Serum creatinine values were normal in all patients. GFR was measured in 10 of the patients. The lowest value 71 ml/min was recorded in patient 3. The intravenous pyelogram was normal in all except patient 14 who had a left-sided kidney stone. None of the patients had proteinuria or signs of endocrine hyperfunction.

Patients 1-9 were treated with equipotential doses of bendroflumethiazide, hydrochlorothiazide and chlorthalidone for 2 weeks with each drug. Patients 10-18 were treated with equipotential doses of triamterene, furosemide and bumetanide for 2 weeks with each drug. The patients were randomized as shown in Table II. The treatment period was preceded by a one-week control period. 24-hour urine samples were collected throughout the control and treatment periods. Specimens of the 24-hour collections were kept frozen until required for analysis. Between each treatment period of 2 weeks there was one week without any treatment.

Serum zinc was determined on the first and last day in every period. Patients were told to avoid alcoholic beverages during the trial, otherwise there were no dietary restrictions. The zinc analyses were performed by atomic absorption (Varian Techtron Model 1100).

Student's paired *t* test was used in the statistical calculations.

Urinary zinc excretion diuretics
J Clin Med 208:209-1980

The importance of zinc in human metabolism has attracted increasing attention since Prasad et al. initially described a zinc deficiency syndrome (11). Zinc deficiency may arise in several

Severe nutritional deficiency leading to kwashiorkor and hypogonadism has been observed in India (11) and Egypt (12). Mild nutritional deficiency in children causing reduced growth has been reported from the USA (18). Alcohol consumption leads to increased urinary zinc losses (14, 15) and the zinc content of the liver is very low in patients with cirrhosis (17). In certain renal diseases such as diabetes mellitus (2) and after surgical injury to the kidney, urinary zinc excretion is raised. Certain endocrine disorders and malabsorption, especially acrodermatitis enteropathica (6, 7), lead to zinc deficiency. In total parenteral nutrition, zinc deficiency is induced by Zn supplementation (3). Increased urinary zinc excretion may also be iatrogenic. Raised urinary zinc excretion has been reported during treatment

Table I Clinical data on the patients subjected to the study

Pat no	Age (y)	Sex	BP at visit	Heart size (ml/m ² BSA)	S-creatinine (mg/100 ml)
1	62	♂	190/130	670	1.2
2	47	♂	210/125	520	0.7
3	53	♀	185/115	320	0.6
4	74	♀	240/130	440	1.1
5	34	♂	195/120	380	1.0
6	56	♀	180/105	430	0.7
7	49	♀	180/115	400	0.6
8	75	♂	200/110	390	1.1
9	69	♂	190/110	400	1.2
10	62	♂	220/110	480	1.1
11	69	♂	190/110	400	1.2
12	53	♀	220/120	440	0.9
13	69	♂	180/110	340	1.0
14	46	♂	220/120	410	1.2
15	46	♀	190/115	360	0.9
16	43	♂	180/130	380	1.1
17	69	♀	220/110	330	1.0
18	67	♂	220/120	400	0.9

RESULTS

The urinary zinc excretion in patients 1-9 before and during treatment with bendroflumethiazide, chlorthalidone and hydrochlorothiazide is presented in Table III. The pretreatment values are mean values of 7-24 hour collections and the values for treatment periods are mean values of 14-24 hour collections. A highly significant increase in urinary zinc excretion of about 300 µg/day was observed during treatment with all three drugs. The zinc concentration in the urine also increased but not to the same extent. During treatment with bendroflumethiazide and hydrochlorothiazide the concentration increased by 1.6 mmol/l ($p < 0.01$). The increase in concentration during treatment with chlorthalidone was 1.8 mmol/l ($p = 0.001$).

Table IV presents the urinary zinc excretion be-

fore and during treatment with bumetanide, furosemide and triamterene. Significant zinc excretion of about 100 µg were obtained during treatment with all three drugs. This was smaller than that during treatment with the thiazides and no increase in zinc concentration was seen. On the contrary, lower though not significantly lower zinc concentrations were found during treatment with furosemide and bumetanide. Serum zinc was within the normal range in all patients before treatment and in all (who successively developed hypotension) during treatment with the different diuretics. The values of serum zinc after treatment with different diuretics did not differ significantly from each other or from the mean pretreatment value.

DISCUSSION

None of the patients in the present study were alcoholics or had diabetes, proteinuria, hypertension or any other known reason for decreased urinary zinc excretion. The 24-hour zinc excretion in the 18 untreated hypertensive patients agrees fairly well with earlier findings of untreated hypertensive patients (1) and of normotensive subjects (4). Serum zinc values were also within the normal range in all untreated patients.

During treatment with equipotential bendroflumethiazide, chlorthalidone and hydrochlorothiazide a highly significant increase in zinc excretion was observed. This was about the same for all three diuretics: bendroflumethiazide and hydrochlorothiazide 65% (Table V). The increased zinc excretion during treatment was partly due to an increase in zinc concentration in urine (31-35% (Table V)) and partly due to an increase in urine volume. About the same increase in urine

Table II Randomization of the treatment

Pat no			
1 2 3	Bendroflumethiazide	Hydrochlorothiazide	Chlorthalidone
4 5 6	Hydrochlorothiazide	Chlorthalidone	Bendroflumethiazide
7 8 9	Chlorthalidone	Bendroflumethiazide	Hydrochlorothiazide
10 11 12	Triamterene	Furosemide	Bumetanide
13 14 15	Furosemide	Bumetanide	Triamterene
16 17 18	Bumetanide	Triamterene	Furosemide

III Mean urinary zinc excretion before and during treatment with bendroflumethiazide, chlorthalidone and hydrochlorothiazide

During 7 days without medication (mg/24 h) (mmol/l)		During 14 days treatment with					
		Bendroflumethiazide (mg/24 h) (mmol/l)		Hydrochlorothiazide (mg/24 h) (mmol/l)		Chlorthalidone (mg/24 h) (mmol/l)	
0.71	6.9	1.17	10.6	0.73	6.5	0.89	7.5
0.76	12.5	1.38	13.1	1.47	12.3	1.81	14.9
0.76	2.6	0.32	3.6	0.34	5.4	0.30	4.0
0.18	1.7	0.53	4.1	0.31	3.5	0.54	5.9
0.40	3.5	0.47	2.3	0.95	5.7	0.59	3.7
0.57	5.7	0.75	7.5	0.67	7.0	0.66	7.3
0.51	5.2	0.80	6.8	0.81	6.9	0.84	7.1
0.46	3.4	0.83	6.0	0.93	6.8	0.81	5.8
0.48	5.5	0.70	7.3	0.72	7.0	0.65	7.6
SD 0.48±0.19	5.2±3.2	0.77±0.33	6.8±3.4	0.77±0.35	6.8±2.4	0.79±0.42	7.0±3.1

During treatment with chlorthalidone was found in an earlier study (20) and this increased after half a year's treatment (21). Pak et al. (22) investigated urinary zinc excretion in 8 subjects during treatment with hydrochlorothiazide and found a zinc augmentation of the same order of magnitude which persisted after one month of treatment. In the present study no differences in zinc increment were observed between patients who started with a particular drug and those who were treated with the same drug at the end of the study.

In contrast to the thiazides the loop-diuretics caused a diminished concentration of zinc in urine during treatment (Table V). Nevertheless the total amount of zinc excreted increased somewhat due to the large urine volumes produced.

Thus the renal handling of zinc is influenced differently by thiazides and loop-diuretics and in an opposite direction compared to calcium. Steel (13) found that the zinc concentration in urine diminished considerably during the first two hours after intravenous injection of furosemide and etacrynic acid but that the total amount of zinc excreted increased. After isotonic saline loading he also noticed an increased zinc excretion and concluded that zinc excretion bears at least some relationship to urine flow. During treatment with triamterene the zinc concentration in urine did not change in the present study but the urine volumes and thus the total excretion of zinc increased somewhat.

Thus during diuretic treatment, especially with thiazides, the amount of zinc lost in the urine is raised and this effect persists during long term

IV Mean urinary zinc excretion before and during treatment with bumetanide, furosemide and triamterene

During 7 days without medication (mg/24 h) (mmol/l)		During 14 days treatment with					
		Bumetanide (mg/24 h) (mmol/l)		Furosemide (mg/24 h) (mmol/l)		Triamterene (mg/24 h) (mmol/l)	
0.39	6.1	0.67	4.9	0.36	4.4	0.48	5.8
0.50	5.5	0.52	4.4	0.51	4.5	0.65	7.5
0.36	5.7	0.40	4.8	0.37	3.6	0.38	5.4
0.35	5.4	0.37	4.7	0.38	5.1	0.39	6.7
0.76	10.2	0.80	12.6	0.95	12.3	0.79	7.0
0.58	6.2	0.75	6.8	0.90	9.9	0.66	7.3
0.73	6.1	0.71	4.8	0.64	4.3	0.75	5.2
0.47	7.9	0.49	4.2	0.49	4.8	0.47	5.0
0.33	4.3	0.36	3.5	0.36	3.5	0.64	7.7
SD 0.49±0.17	6.4±1.7	0.56±0.17	5.6±2.8	0.55±0.23	5.8±3.4	0.58±0.15	6.4±1.1

Table V Change (%) in urinary zinc excretion between values observed without and with treatment with six diuretics

	Amount	Concentration
Bendroflumethiazide	60	31
Chlorthalidone	65	35
Hydrochlorothiazide	60	31
Bumetanide	14	-14
Furosemide	12	-13
Tiamterene	18	0

treatment. In spite of this fact the serum zinc concentration is normal or even slightly raised during diuretic therapy but if this is dependent on increased zinc absorption or tissue zinc depletion remains to be clarified. It is also unclear if the increased urinary losses of zinc will lead to a zinc deficiency of clinical significance. However since diuretic treatment is very common and zinc has been recognized as an essential element for human health the diuretic-zinc problem calls for further attention.

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Electrocardiographic Diagnosis of Ventricular Septal Infarction

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ABSTRACT To find electrocardiographic criteria for ventricular septal infarction, two series of ECGs were studied: all without fascicular/bundle branch block and complete heart block. One series consisted of ECGs recorded at thallium 201 scintigraphy in patients 2-3 weeks after an acute myocardial infarction (AMI) in this series the 14 patients with a defect in the septal wall of the left anterior oblique coronary artery were compared with the 35 without. The other series consisted of the last pre-mortem ECGs in 20 AMI patients with and in seven without a septal involvement of the infarct at autopsy. The best criterion for earlier literature was absence of a q wave in lead V₁, showing a sensitivity of 53% in the combined scintigraphy and autopsy series. The predictive value of a positive test was 75%. The very first QRS deflection in the frontal plane tended to discriminate better than absence of a q in lead V₁. With a similar relative value 71% the sensitivity of a deviating vector (+120° in -60° and -120° to -180°) was 65%. In the combined scintigraphy and autopsy groups 10 of the patients with a deviating vector showed myocardial infarction. The clinical importance of an early diagnosis of septal involvement in AMI remains to be

not to produce any specific ECG pattern (5). Today septal infarcts can be detected in vivo by thallium 201 scintigraphy and one can reassess the problem of diagnosing them on ECG.

The aim of this study was to compare the ECGs in patients with and without septal involvement of a myocardial infarct according to thallium 201 imaging in one series and to autopsy in another. Three ECG patterns were suggested many years ago as indicating septal involvement: qrs complexes in leads V₁ (4), QS in V₁ (6) and disappearance of the normal q wave (3). As the ventricular activation normally starts in the septum, it was also considered worthwhile to study the frontal vector of the first QRS deflection.

PATIENTS AND METHODS

Thallium 201 scintigraphy series

Fifty-five non-consecutive patients with acute myocardial infarction (AMI) were studied before discharge from hospital, usually during the second or third week after the infarction. The main selection mechanism was that a thallium 201 dose was available during the last days before discharge. Fifty-one males and four females were included, aged 37-79 years (mean 58.3). Twelve of them had a history of previous AMI. The current AMI was diagnosed on the basis of history, serial ECG and serum enzymes (CK, ASAT, ALAT, LDH). The ASAT maximum was 0.8-14.3 median 2.9 µkat/l (upper normal limit 0.7).

In 50 of the 55 patients the dose 75 MBq thallium 201 was injected in vivo during exercise on a bicycle ergometer; in the other five this was done at rest. Imaging was started 10 min after the injection, using a Nuclear Chicago Pho Gamma camera with high resolution collimation. Polaroid scintiphotos (400 000 counts) were obtained in the anterior, 45° left anterior oblique (LAO) and left lateral

Keywords: ventricular septal infarction, electrocardiography, myocardial scintigraphy.
Acta Med Scand 208 213 1980.

The standard electrocardiogram (ECG) does not indicate the precise location of a myocardial infarct in the left ventricle (8) but it is reliable in distinguishing infarcts involving the anterior from those involving the inferior/posterior wall (5) which has a prognostic implication (2). From the prognostic point of view the ventricular septum might well be the most important wall as it influences the pump function of the ventricles and carries the main parts of the ventricular conduction system. Based on autopsy studies septal infarcts are however stated

Abbreviations: ECG = electrocardiogram; AMI = acute myocardial infarction; CCU = coronary care unit; LAO = left anterior oblique.

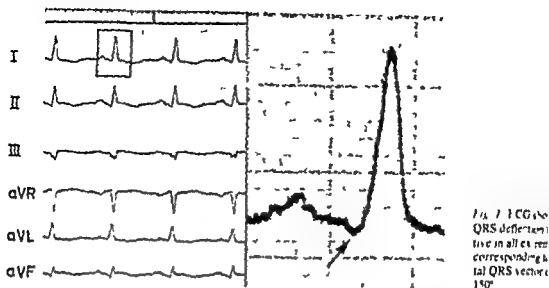


Fig. 1 ECGs No QRS deflection in all extremity leads corresponding to a frontal QRS vector 150°

projections. The imaging was repeated 2–4 hours later if a defect was detected in any projection at the immediate imaging. These second images together with those after injection at rest were used for the correlation to various patterns of a 12-lead ECG taken at rest immediately before the injection procedure. All images were studied for defects in the septal wall in the LAD projection by two observers working independently. The defects were classed as definite or probable.

Autopsy series

Thirty-seven consecutive autopsied AMI patients were collected from the files of the coronary care unit (CCU) and the post-CCU ward. Twenty-four were males and 13 females, aged 47–85 years (mean 64.6). Sixteen of them had a history of previous AMI. The ASAT maximum of the current AMI was 1.2 – 12.6 median 6.4 $\mu\text{kat/l}$. The interval between onset of AMI symptoms and death was 1–71 days (mean 7.3). The most common causes of death were shock 16, rupture 5, heart failure 3, and unexpected ventricular fibrillation/asystole 5. A routine autopsy was performed. The last pre-mortem ECG 0–13 days (mean 1.9) before death without complete heart block and fascicular/bundle branch block was used for correlative studies.

The ECGs from the scintigraphy and autopsy series were studied for fascicular block, bundle branch block, complete heart block, and artificial pacing. If none of these was present, QRS configuration in leads V_1 or V_2 or QS in V_3 or V_4 was noted as was the absence of q in V_6 . The frontal vector angle was also determined based on the first QRS deflection in the six extremity leads (Fig. 1) and classified in steps of 30 degrees: 1 – 165° , 135° – 135° , $+135^\circ$ or $+165^\circ$, e.g. a vector of -165° implies between -180° and -150° . For comparison, the initial (0.03 sec) vector was also estimated in steps of 30 degrees.

The ECGs were interpreted by two observers working independently, one of them without any knowledge of the scintigraphy or outcome of the autopsy. In the event of different interpretations of ECGs or scintigrams, a third observer's opinion was decisive.

The exact treatment was used instead of the fourfold tables (1).

RESULTS

Thallium 201 scintigraphy series

At the time of scintigraphy, no patient had heart block, but six had fascicular or bundle branch block. Only one of these six patients had a septal image defect.

Of the remaining 49 patients, 10 showed septal image defect and four a probable QRS configuration in V_1 or V_2 nor a QS or in V_3 or V_4 was significantly more common in patients with than without definite septal image defect, but this was the case for absence of a q in V_6 (Table 1). The sensitivity for absence of q in V_6 is however rather low, 50%.

In three patients, all without a septal image defect, the first QRS vector could not be determined unequivocally, probably because the first QRS deflection was so small that it was not discernible in the extremity leads. The distribution of the frontal QRS vector in the remaining 46 cases is presented in Fig. 2. In patients without septal image defects, the frontal QRS vector was predominantly at -105° , -75° , $+135^\circ$ and $+165^\circ$. Other vectors as deviant, their incidence was significantly higher in patients with than without definite septal image defects, i.e. $8/10$ against $1/16$. The sensitivity tends to be higher than for absence of q in V_6 , 80% against 50%, and the predictive value of a positive test is similar, 63% and 46% respectively. Ten of the 16 patients with a definite

1 ECG diagnosis of the frontal plane series and the apical series

	Thallium scintigram series			Autopsy series			
	Definite septal defect (n=10)	Probable septal defect (n=4)	No septal defect (n=35)	Large septal infarct (n=7)	Small septal infarct (n=7)	Imprecise septal infarct (n=6)	No septal infarct (n=7)
V ₁	30	0	6	14	0	33	29
V ₂	40	5	11	14	0	11	14
mV	50	5	11*	46	43	50	9
in frs QRS ecto (13 to 45 to 105°)	80	50	n=3	86	43	50	9

*Tandifference ($p < 0.05$)

with the first ECG and in the remaining developed during the first 15 days in hospital. The vector was not associated significantly with anterior or inferior/posterior site of infarct according to ECG although anterior were over-represented in the group with septal defects ($p < 0.05$).

First QRS vector was not correlated to the frontal QRS vector ($r = 0.10$). This first QRS vector was also compared to the so-called normal (sec) QRS vector in the frontal plane in 43 patients. They corresponded in only four patients usually differed 30° – 90° .

Septal infarcts are stated to be detectable also in medial wall in the anterior scintigraphic projection but the first QRS vector of patients with a defect in the upper part of that wall was distributed like the rest of the patients. The same holds true in the lower part of the medial wall in the frontal projection. Thus only the LAO projection in infarcted patients with different vectors.

Two observers differed regarding a septal

LAO defect in five of the 55 scintigrams. There was no disagreement regarding the QRS in V₁ or in V₂ or the first QRS vector.

Two of these 49 patients without complete heart block or fascicular/bundle branch block at scintigraphy had a temporary complete heart block for some time during the hospital stay and two showed temporary bundle branch block. One of these four patients had a septal myocardial defect.

Autopsy series

A septal involvement of the infarct definite or probable was much more common in the deceased according to autopsy (73%) than in the survivors according to the LAO projection in the thallium-201 scintigram (77%) ($p < 0.05$).

In 10 of the 37 autopsied patients all ECG showed complete heart block or fascicular/bundle branch block and could not be evaluated with regard to the various QRS patterns studied (3 of 10 with large 3 of 10 with small 1 of 7 with imprecise and 3 of 10 without septal involvement). Absence of

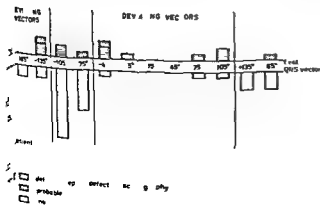


Fig. 2. Distribution of the first QRS vector in patients with septal myocardial defect in the LAO projection (upper part) and without such defect (lower part).

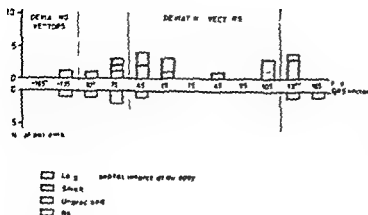


Fig. 3 Distribution of the first QRS vector in patients with septal infarct (upper part) and without septal infarct (lower part)

q in lead V_4 was more common in patients with large than with no septal involvement ($p=0.05$) (Table 1).

The first QRS vector could be determined in all 27 patients without complete heart block or fascicular/bundle branch block (Fig. 3). Patients with large or no septal involvement at autopsy are distributed similarly to those with definite or no septal image defect at scintigraphy. Deviating vectors i.e. others than -105° , -75° , $+135^\circ$ and $+165^\circ$ were found in six of the seven patients with large septal infarcts and in only two of the seven without septal involvement ($p=0.05$). The groups with small and imprecise septal infarcts were in between (Table 1).

Eight of the 27 patients without fascicular/bundle branch block or complete heart block in all ECGs had a temporary complete heart block and they were rather evenly distributed in the four subgroups. Two of these seven patients and six others showed temporary bundle branch block and there was no dominance for any subgroup in this respect.

The two observers did not differ regarding the occurrence of qrs in V_1 , QS in V_4 , or q in V_4 , but in three cases the vector differed one step (30°).

DISCUSSION

A septal involvement of the infarct was found more frequently in the autopsy series (73%) than in the scintigraphy series (27%). This difference may have several explanations. The infarcts of the survivors were smaller as measured by S-ASAT ($p<0.05$) and hence a detectable septal involvement is less likely. Furthermore, a small septal infarct may pass undiagnosed more easily at scintigraphy than at autopsy. In this series the scintigraphic diagnosis of a septal infarct was made only in the

LAO projection and excluding the artefaction may have given some false negative results. However, as defects in the anterior wall in LAO position did not discriminate between different first QRS vectors, the artefaction appears to reveal only a small fraction of infarcts or infarcts so located e.g. high in the wall are not reflected by the very first part of Q.

According to experimental studies (1) by Scher (7), the very first QRS activation is superiorly, rightward and anteriorly. This corresponds well to first QRS vector in the form of -105° which was found in 14 of the 27 without a demonstrable septal infarct (Fig. 3). Eight of the 39 had however a first QRS of $+135^\circ$ or $+165^\circ$ and that was not due to different heart position as measured by the QRS axis. Nor did they show a more or less defect high in the medial wall in the anterior wall.

The interpreter variation in determining the first QRS vector was small, only 4% of the differed between two independent observers and only one step (30°). The sensitivity of a first vector is acceptable (65% for all infarcts in combined scintigraphy and autopsy series). The predictive value of a positive test (100% in combined series). The predictive value for absence of q in V_4 is 55%, but the sensitivity tends to be less (53%).

It could be discussed if a vector between -120° to -180° should be considered as not. In this sector there were also some patients with signs of septal infarct and small (Figs. 2+3). Considering those vectors as not reduces the sensitivity to 53%, but the predictive value of a positive test increases to 75%.

c to 82% which should be compared to 75% absence of q in V_4 .

It seems reasonable that the first QRS vector should show a better sensitivity than qrs in V_1 , QRS r_{S1} and absence of q in V_4 . These patterns are confined to the first csec of QRS when the nuclear activation starts in the septum. Analogously this first QRS vector differs from the so-called alone (0.04 sec).

The clinical importance of early diagnosis of a septal involvement remains to be settled although an echocardiographic study of anterior infarction showed septal involvement to be correlated to development of heart failure and bundle branch

block (9). In the present retrospective series the effect on pump function of a septal involvement in infarction cannot be studied as all patients were treated simultaneously with rather high doses of diuretics. The incidence of complete heart block did not differ between patients with and without septal involvement in the present combined scintigraphy-autopsy series (16% and 28% respectively). Bundle branch block however was significantly more common in patients with septal involvement (22% against 6% $p < 0.05$).

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Medical Association against Heart and Chest Diseases.

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Thallium-201 Scintigraphy after Acute Myocardial Infarction

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OBJECT Fifty-five patients with acute myocardial infarction were examined with thallium 201 scintigraphy before discharge from hospital. Fifty-one significant scintigraphic defects. The number of normal Q waves in a 12-lead ECG but not the normal ASAT value for each patient were correlated to the total estimated image defect. Mortality in the follow-up period of 14-28 months was not related to the estimated total image defect but the early deaths occurred among patients with the dilated left ventricles.

KEY WORDS thallium 201 scintigraphy, acute myocardial infarction.

Acta Med Scand 208 219 1980

The remaining amount of viable left ventricular myocardium and its functional state are reasonably important prognostic factors following an acute myocardial infarction (AMI). Serum enzyme maxima during the acute phase give a rough estimate of the extent of the current infarct. Cold spot myocardial scintigraphy can give information about the myocardial loss in current and previous AMI. Myocardial ischaemia of the remaining myocardium can be evaluated at the same time by injecting thallium-201 (²⁰¹Tl) during exercise and imaging immediately and after 4 hours (6-8). The scintigram provides some information about left ventricular function.

Thallium-201 scintigraphy has been used following AMI. In a recent multicenter study of 145 AMI patients during the hospital stay (7) concluded that the scintigraphy complements the electrocardiographic identification of the infarct. The present study concerns another aspect: what does ²⁰¹Tl myocardial imaging add to the information that can

be obtained about the remaining amount of viable myocardium and its functional state from history, ECG, serum enzyme maxima and chest X-ray?

PATIENTS AND METHODS

Fifty-five consecutive AMI patients, 51 males, 4 females, aged 37-79 years (mean 58.3) were studied before discharge from hospital, usually during the second or third week after an infarction. The main selection mechanism was that a ²⁰¹Tl dose was available during the last days before discharge. Twelve patients had a history of previous AMI. The current AMI was diagnosed on the basis of history, serial ECG and serum enzymes (CK, ASAT, ALAT, LDH). The ASAT maximum was 0.8-14.3, median 2.9 μ kat/l (upper normal limit 11.7). Twenty of the infarcts were anterior (ANT), 27 diaphragmatic and four lateral according to serial ECG; four were not localizable.

A chest X-ray and ²⁰¹Tl scintigraphy with injection during exercise were performed before discharge. The heart X-ray was taken in the standing position and the heart volume was calculated (4). The upper normal limit used were 500 ml/m² BSA for males and 450 ml/m² BSA for females. Radiological heart volume is not available in four patients.

For the scintigraphic examination 75 MBq ²⁰¹Tl was injected intravenously during exercise in the sitting position on a bicycle ergometer at 60 of the 55 patients. The injection was given about 2 min before termination of the exercise on a load of 40-160 W (median 60). Most bicycle tests were discontinued before the patients had developed any symptoms. Because of heart failure the remaining five patients were injected at rest in the standing position to reduce splanchnic blood flow and thereby uptake by the liver.

Imaging was started 10 min after the ²⁰¹Tl injection. Images were obtained in the ANT, 45° left anterior oblique (LAO) and left lateral (LL) projections. Imaging was re-

Abbreviations ²⁰¹Tl = thallium 201, AMI = acute myocardial infarction, ECG = electrocardiogram, electrocardiographic, ANT = anterior, LAO = left anterior oblique, LL = left lateral.

Table 1 Estimated degree of left ventricular dilatation in scintigrams of radionuclide left ventricles at rest and after exercise

Degree of scintigraphic left ventricular dilatation	Radionuclide heart volume (ml/m ² BSA)		
	Normal	Moderate increase (<100)	Marked increase (>100)
At rest	18	6	0
After exercise	10	6	0
At rest and after exercise	3	3	5

(9-18 months later) were scattered among the types presented in Table 1.

DISCUSSION

If patients without evidence of prior AMI in a center study (7) 81% had ^{201}Tl image defects at hospitalization for AMI. In the present series the corresponding figure was 91%. A similar incidence of new abnormal Q waves (64 and 68% respectively) speaks in favour of a similar average size in the two series. The difference in the frequency of positive ^{201}Tl scintigrams (81 against 1) may be due to differences in the criteria for a significant image defect. An infarct must have a minimum size to be detectable by ^{201}Tl scintigraphy according to both experimental and clinical experience. (5) The location of the infarct may also be relevant. In this series no defect in the posterior wall fulfilled the criteria of a significant defect. The total number of abnormal Q waves correlates well to the image defect score, but the scatter is large. Abnormal Q waves after AMI may disappear with time (7) and the criterion of a Q wave 1 sec does not detect more than 61% of infarcts diagnosed at autopsy (3). However, ^{201}Tl scintigraphy correlates with autopsy in a large clinical trial remains to be seen. The exercise test was performed with caution, as usually terminated before symptoms or ST-segment depression appeared, neither the ECG nor the scintigrams are fully conclusive with regard to the presence of ischaemia. Redistribution ^{201}Tl scintigrams after exercise seem, however, to have a higher sensitivity for myocardial infarcts than scintigrams after injection at rest (1). The conclusion from this series suggests that 1) ^{201}Tl

scintigraphy 2-3 weeks after an AMI demonstrates defects in most cases, reflecting the current infarct and probably also undiagnosed prior infarcts. Mortality during the following 14-78 months is, however, not related to the estimated total image defect. Studies of the patients' functional state after the hospital phase in relation to the remaining amount of viable myocardium are needed. (2) ^{201}Tl scintigrams performed after injection during exercise give some information about ischaemia in the remaining viable myocardium. Image defects that diminished or disappeared in 2-4 hours were seen in about one third of the patients. (3) ^{201}Tl scintigrams offer a possibility of a rough estimation of left ventricular dilatation, which seems to be related to short-term survival after the hospital phase.

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Effect of Metoprolol on QT_c Intervals after Acute Myocardial Infarction

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FRACT The effect of metoprolol on corrected QT interval (QT_c) was studied retrospectively in 111 patients of AMI below 70 years of age. Prior to randomization the patients were stratified by age, infarct size and ventricular arrhythmias and randomized to metoprolol, 100 mg b.i.d. or placebo were double-blindly to 59 and 52 patients, respectively. QT_c intervals were measured four times prior to randomization and three times during the follow-up. The highest QT_c mean was registered on the 1st day in the CCU. QT intervals subsequently decreased significantly in both groups between discharge and the three month control ($p < 0.001$). Patients on metoprolol had significantly shorter QT_c intervals during the follow up year than those on placebo (0.394 ± 0.028 vs 0.406 ± 0.034 sec, $p < 0.01$). The QT_c shortening effect of β receptor blockade was most marked in patients with prolonged QT_c intervals at discharge. Patients who died prior to discharge had prolonged QT_c intervals prior to discharge. In this group the proposed beneficial effect of β receptor blockade on QT_c interval cannot be studied as most of these patients had died before randomization control.

on QT interval acute myocardial infarction
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Acta Med Scand 208 223 1980

is with previous myocardial infarction (MI) constantly prolonged corrected QT intervals (>440 msec) have been found to run a higher risk of dying suddenly (21). Furthermore survivors of hospital ventricular fibrillation (VF) have been found to have longer QT_c intervals than other patients with post MI patients (10). We found that patients with AMI who developed ventricular tachycardia (VT) or VF in the acute phase have longer QT_c intervals on admission (4). Further QT interval prolongation at discharge from hospital may also predict future cardiac events and

sudden deaths within the first six months in patients with AMI (3).

As a beneficial effect of treatment with β receptor blocking agents has been reported on survival in AMI patients (14-26) and as these drugs seem to shorten the QT_c interval (15-22-24) we decided to analyse the effect of long term β receptor blockade on QT_c intervals during a one year follow up of AMI.

PATIENTS AND METHODS

To be included in the study the patients had to be below 70 years of age, to be in sinus rhythm at discharge and to show no evidence of complete bundle branch block, severe heart failure, hypotension or bronchial asthma. From May 1977 to Dec 1978 111 consecutive patients with AMI were discharged alive from the hospital and included in the study. There were 88 men, mean age 60 years (range 44-69) and 23 women, mean age 58 years (range 46-69). Details concerning catchment area and routines of the unit have been published elsewhere (6). Salient clinical data were registered by code on special charts and transferred to punch-cards for computer analysis (9). Prior to discharge the patients were stratified and randomized. Placebo or metoprolol 100 mg b.i.d. were given double blindly to 52 and 59 patients, respectively.

Stratification was based on 1) Infarction size estimated by enzyme maximum of LD₁ (thermostable fraction LD₁ and LD₂ of lactodehydrogenase) LD₁ <20 μ kat/l small infarction LD₁ ≥ 20 μ kat/l = large infarction 2) Type of ventricular arrhythmias registered on a six hour ECG three hours at day time with moderate exercise three hours at night time (16) 3) Age (below 65 years and 65 years or more).

Abbreviations QT=corrected QT interval MI=myocardial infarction AMI=acute MI VF=ventricular fibrillation VT=ventricular tachycardia

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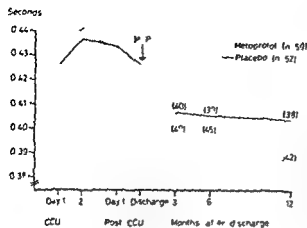


Fig 1 Mean QT_c interval in patients given metoprolol or placebo after AMI

After discharge the patients were seen regularly in the Out Patient Clinic. They were readmitted for six hours ECG recording and laboratory investigations 6 and 12 months after the acute event. End points were reinfarction or sudden death.

Seventeen patients were withdrawn from the study due to side-effects or a need of β blockade to control deteriorating angina pectoris. Of the ten patients who died during the follow-up year seven (3 on metoprolol 4 on placebo) died suddenly; five of them within three months. One of the patients who died suddenly had suffered a reinfarction prior to death. One patient died of lung cancer. Nine patients (4 on metoprolol 5 on placebo) suffered ten reinfarctions fatal in two cases. In six of the patients the reinfarction occurred within three months.

The QT_c intervals were measured retrospectively by one of us (S.A.) without knowledge of the patients' clinical data. QT_c intervals were calculated on seven occasions: on the first two days in the CCU, on the first day after discharge from CCU, at discharge from hospital prior to randomization, and at 3, 6 and 12 months after the reinfarction. From a 12 lead resting ECG recorded at a speed of 50 mm/sec the QT and RR intervals were measured in 3-5 consecutive beats. These values were averaged and the QT was calculated from the mean according to Bazett's formula (5, 13):

$$QT_c = \frac{QT}{\sqrt{RR}}$$

The measurement was made in the lead with the longest QT interval. Care was taken not to measure intervals preceded by premature beats or to include U waves in the QT interval. The QT interval was measured in hundredths of a second from the beginning of the QRS complex to the end of the T wave where its hind limb joined the base line. Differentiation between T and U waves or some other potential was usually possible in at least one lead. If this was not possible the QT interval was measured from the beginning of the QRS complex to the notch between the T and U waves (13).

Definitions

Acute myocardial infarction was diagnosed; conventional criteria (1-18). Left heart failure, noted by the presence of basal pulmonary monary vascular congestion on X ray. Inferior if the ECG criteria for infarction were in two or more of leads V₂-V₄, lateral if in two or more aVL, V₅ and V₆ and inferior if in two or more III and aVF. Combination sites were determined to the same criteria. In the following anterolateral sites are classified as anterior lateral sites as inferior. Sudden death was defined within two hours after onset of the final symptoms. Endocardial infarction: ST-T changes only with enzyme pattern. Transmural infarction: 40-70% pathological Q wave (18) in at least two of 12.

Statistical methods

Conventional statistical methods were used. Significance of differences between the mean QT_c patient group was tested by Student's *t*-test.

RESULTS

QT_c interval in relation to time elapsed AMI and to metoprolol therapy (Fig 1)

The longest QT_c mean was registered second day in the CCU, whereafter the QT_c values decreased. QT_c intervals did not change significantly on any occasion during the period between patients subsequently given metoprolol or placebo. QT intervals decreased significantly in both treatment groups between discharge and the three month control. Throughout the follow-up period patients on metoprolol had significantly shorter QT_c intervals than those on placebo (0.394±0.0406±0.034 sec (mean±SD), *p*<0.001; in the metoprolol group there was a further decrease in QT_c intervals between the 3 and 12 month follow-up (0.398±0.025 vs 0.387±0.024 sec), which was not seen in the placebo group.

As expected the heart rate decreased significantly in the metoprolol group between discharge and the 3 month follow-up (74±16 vs 61±9 beats/min, *p*<0.001) while it remained constant in the placebo group (73±14 vs 73±13 beats/min). Between 3 and 12 month follow-up a further decrease in heart rate was observed in the metoprolol group (67±5 vs 56±6 beats/min, *p*<0.05) but not in the placebo group (73±13 vs 69±13 beats/min, NS).

1 Mean QT_c interval (sec) in relation to type and site of infarct

metoprolol P = placebo

		Before drug (days)						On drug (months after discharge)		
		No of pts	CCU		Post CCU		p	3	6	12
Drug			1	2	1	At discharge				
Myocardial infarct										
anterior	M	48	0.432	0.444	0.430	0.423	<0.001	0.400	0.398	0.385
	P	38	0.425	0.438	0.434	0.431	<0.01	0.406	0.403	0.403
subendocardial	M	11	0.432	0.440	0.433	0.405	N.S.	0.385	0.400	0.390
	P	10	0.424	0.441	0.430	0.422	N.S.	0.413	0.429	0.420
transmural	M	5	0.436	0.424	0.436	0.405	N.S.	0.377	0.397	0.400
	P	4	0.438	0.413	0.445	0.460	N.S.	0.400	0.387	0.383
Site of infarct										
anterior	M	22	0.433	0.445	0.435	0.423	<0.05	0.398	0.395	0.388
	P	22	0.422	0.443	0.442	0.437	<0.05	0.409	0.408	0.412
non-anterior	M	26	0.432	0.445	0.427	0.420	<0.01	0.399	0.397	0.393
	P	19	0.426	0.428	0.423	0.425	N.S.	0.409	0.407	0.407
inferior	M	11	0.429	0.427	0.431	0.414	N.S.	0.394	0.405	0.393
	P	11	0.434	0.440	0.436	0.432	N.S.	0.399	0.404	0.386

Significant difference between 3- 6- and 12 month control unless otherwise stated

*p<0.05

2 site and size of infarct and complications

own in Tables I and II QT_c intervals decreased similarly between discharge and the 3 month control in the various subgroups. The decrease in QT_c however was more pronounced and reached a higher level of significance in relation to the parameters of the metoprolol group i.e. anteriorly inferior or small infarcts (LD, <20 mm), left heart failure and in the subgroup with infarct in the CCU.

3 Arrhythmias

There was no significant association between QT_c intervals and the incidence and type of ventricular premature beats as registered by telemetry prior to hospitalization or at the 6 and 12 month control in the metoprolol or placebo group.

4 Changes in QT_c intervals in relation to QT_c at discharge (Table III)

Patients with short QT_c intervals (≤0.420 sec) at discharge showed no further significant decrease in QT_c registered at the 3 month control. In contrast a significant decrease between these two occasions was registered in patients with intermediate QT_c

intervals (0.430-0.440 sec) in the metoprolol group but not in the placebo group. In patients with long QT_c intervals (>0.440 sec) there was a significant decrease in both groups between discharge and the 3 month control. In the metoprolol treated patients with QT_c intervals above 0.440 sec a further sig-

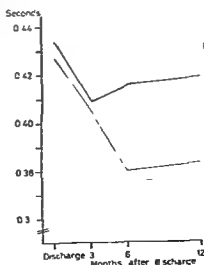


Fig 2 Mean QT interval after AMI in relation to therapy with metoprolol (M) or digitalis (D)

Table II Mean QT_c interval (sec) in relation to size of infarct and CCU complications
M = metoprolol P = placebo

	Drug	No of pats	Before drug (days)						On drug (months after)	
			CCU		Post CCU		p	3	6	
			1	2	1	At discharge				
<i>Size of infarct</i>										
<i>LD₁ < 20</i>	M	39	0.429	0.439	0.429	0.413	<0.05	0.398	0.391	
	P	31	0.425	0.445	0.433	0.426		N.S.	0.410	0.411
<i>LD₁ ≥ 20</i>	M	20	0.438	0.447	0.435	0.434	<0.001	0.397	0.391	
	P	21	0.427	0.425	0.435	0.440		<0.001	0.401	0.394
<i>CCU complications</i>										
<i>Left heart failure</i>	M	37	0.437	0.444	0.433	0.424	<0.001	0.394	0.391	
	P	40	0.431	0.439	0.434	0.431		<0.01	0.409	0.401
<i>No left heart failure</i>	M	22	0.424	0.438	0.426	0.413	N.S.	0.404	0.401	
	P	12	0.408	0.429	0.436	0.433		N.S.	0.400	0.400
<i>VT</i>	M	19	0.416	0.439	0.434	0.420	<0.01	0.390	0.401	
	P	20	0.429	0.434	0.453	0.436		<0.01	0.407	0.401
<i>No such arrhythmias</i>	M	40	0.430	0.443	0.429	0.420	<0.01	0.401	0.396	
	P	31	0.425	0.440	0.422	0.429		<0.05	0.407	0.408

No significant difference between 3-6- and 12-month control

nificant decrease was, however, registered between the 6- and 12-month controls (Table III).

In the metoprolol group a significant decrease was noted in heart rate between discharge and the 3-month control within the three subgroups with QT_c intervals above 0.410 sec ($p < 0.05$, $p < 0.01$, $p < 0.05$). No further significant decrease was seen in these subgroups between the 3- and 12-month control. No significant change in heart rate was registered in the placebo group.

QT_c intervals in patients with or without digitalis therapy (Fig. 2).

Thirty patients in the metoprolol group and placebo group were not on digitalis at discharge during the follow-up. The corresponding number of patients on digitalis during this period was 11. The remaining patients were on digitalis for shorter periods or intermittently.

QT_c intervals decreased significantly between discharge and the 3-month control in pa-

Table III Mean QT_c interval (sec) in subgroups within different QT_c limits at discharge
M = metoprolol P = placebo

QT at discharge	Drug	No of pats	Before drug at discharge	p	On drug (months after discharge)		
					3	6	12
<0.400	M	18	0.384	N.S.	0.391	0.382	0.384
	P	14	0.390	N.S.	0.395	0.395	0.395
0.410-0.420	M	14	0.411	N.S.	0.392	0.399	0.399
	P	10	0.414	N.S.	0.405	0.403	0.403
0.430-0.440	M	14	0.434	<0.01	0.404	0.404	0.404
	P	11	0.436	N.S.	0.422	0.416	0.416
>0.440	M	13	0.464	<0.001	0.401	0.406	0.406
	P	17	0.473	<0.001	0.408	0.417	0.417

No significant difference between 3-6- and 12-month control unless otherwise stated

* $p < 0.05$

metoprolol with or without digitalis ($p < 0.01$). This was also for patients without digitalis in the group ($p < 0.05$). During the follow-up patients on both metoprolol and digitalis had significantly shorter QT intervals than those on metoprolol only (0.378 ± 0.031 vs. 0.404 ± 0.071 s) (0.1). Similarly in the placebo group patients on digoxin had significantly shorter QT intervals during the follow-up (0.390 ± 0.071 vs. 0.414 ± 0.079 s) ($p < 0.01$).

In patients on metoprolol only there was a significant decrease in QT intervals between discharge and 3 month control among patients with mean values of 0.410 ± 0.040 sec and above 0.440 s at discharge (0.434 ± 0.005 vs. 0.413 ± 0.015 s) ($p < 0.05$ and 0.464 ± 0.011 vs. 0.409 ± 0.018 s) ($p < 0.001$ respectively). In the corresponding subgroup this was true for patients with mean below 0.440 sec (0.474 ± 0.071 and 0.419 ± 0.079 s) ($p < 0.01$).

QT intervals in patients with subsequent cardiac events

Patients who subsequently died suddenly had significantly longer QT intervals than those with major cardiac events at discharge and prior therapy (0.453 ± 0.057 vs. 0.473 ± 0.035 s) ($p < 0.05$).

Two of these patients were on digitalis and also on quinidine at discharge.

QT intervals in those 9 who suffered reinfarction terminated at $(0.477 \pm 0.040$ sec) and did not significantly differ from the QT intervals of those who died suddenly or who had an uneventful reinfarction.

Five of the patients who suffered a reinfarction were on digitalis and another on both metoprolol and digitalis.

A statistical analysis was performed at the 3 month control of patients who suffered major cardiac events as most of these events had already occurred within this period.

DISCUSSION

Prolonged QT interval in patients with previous myocardial infarction increases the risk of sudden death (1). In accordance with previous studies (3, 7) we found QTc prolongation during the acute phase of an AMI in our patients and a significant decrease at subsequent measurements. In previous studies there are indications that the severely ill MI patients tend to have longer

QT intervals (2, 3, 4). During the acute phase of an infarction QT prolongation may be due to local hypothermia (8, 13) or hypocalcaemia (13, 15). QT interval prolongation may to some extent also depend on increase in or imbalance of the sympathetic nervous system (70). It is known from animal experiments that ablation or blockade of the left and right stellate ganglion will result in a marked decrease and increase respectively in the AVF threshold (70) and also influence the QT interval. Right stellectomy will prolong the refractory periods on the anterior cardiac wall and left stellectomy mainly on the posterior ventricular wall (71). As QT prolongation seems to be a common feature in MI patients who develop ventricular fibrillation (10) or subsequent sudden death (72) a possible therapeutic approach would be to try to decrease sympathetic tone and hereby shorten the interval. This has indeed been achieved in human results with β blocking agents in the hereditary long QTc syndromes (11, 17, 19, 73). A beneficial effect of β blockade on mortality has been reported in studies of patients after an AMI (14, 74). Possible mechanisms behind this phenomenon are still being discussed and not settled.

Acute administration of β blockers in patients with QT intervals (75, 76). Nyberg et al. (76) entered the same effect of QT interval on short-term therapy with alprenolol. The result was that the decrease in QT interval was pronounced in the most severely ill patients. These patients had the highest mean QTc at treatment. In agreement with the results we found the shortening effect of β blockade on QTc to be most marked in those with prolonged QT intervals at discharge. This reduction cannot be explained by other drugs influencing the QT interval in digitalis (77) as these findings were also obtained in patients not on digitalis.

We also looked at QTc interval changes in relation to type, size and size of infarct. CCU complications and ventricular arrhythmias registered by telemetry prior to randomization and at the 6 and 12 month controls. However, in this prospective study which is still in the sampling stage the number of patients in these subgroups is too small to allow any conclusions about a probable beneficial clinical effect of β receptor blockade or whether such an effect is related to changes in QT intervals.

A finding in a previous study (3) was confirmed

here namely that patients dying suddenly during the first follow up year have longer QT_c intervals at discharge than those without major cardiac events. The proposed beneficial effect of β receptor blockade on QT_c interval in this group of patients can not be evaluated as most of these patients had died before the first control. A report will follow on these patients in a future follow up study.

ACKNOWLEDGEMENTS

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Enzyme Activities in Serum after Extensive Exercise, with Special Reference to Creatine Kinase MB

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TRACT It is well documented that elevations of enzymes used as criteria in establishing the diagnosis of acute myocardial infarction (AMI) often show a pattern of AMI after physical exercise with other clinical signs of myocardial damage. Since a condition resembling AMI sometimes appears after strenuous physical exercise, this study was designed to show if the new almost heart specific isoenzyme creatine kinase MB (CK-MB) would solve diagnostic problems. Ten well trained volunteers participated in a 26 km jogging race. None of them had cardiovascular symptoms, but the old cardiac enzymes rose in some of them above the discriminative levels, whereas CK-MB was below these levels in all. It is concluded that CK-MB determination is a valuable diagnostic tool also in patients who have not exercised extensively.

cardiovascular diseases (8-12) or hypothermia (2) without AMI. Their findings can be explained by the small amounts of CK-MB found in skeletal muscle (4-7). However, these disorders rarely complicate the differential diagnosis of AMI, so they are of minor clinical importance. In contrast, the increasingly popular sport of jogging sometimes involves a clinical condition resembling an AMI (1, 6).

As conclusions based on determinations of serum LD, ASAT and CK after physical exercise are controversial, the pattern found sometimes resembling that of AMI (3, 6), these enzymes are not suitable for ruling out the presence of this disease. The principal aim of the present study was to elucidate whether determination of serum CK isoenzyme could redress this confusion.

Keywords: creatine kinase MB, exercise, acute myocardial infarction, diagnosis.

Med Scand 208 229 1980

SUBJECTS AND METHODS

Ten volunteers completing a 26-kilometer jogging race (Møllegaardet) participated in the study. Their clinical data are presented in Table I. Their level of training is indicated by the number of km they have been running in the month before the race.

Determination of serum lactate dehydrogenase (EC 1.1.1.27 LD), aspartate aminotransferase (EC 2.6.1.1 ASAT) and creatine kinase (EC 2.7.3.2 CK) has been widely used in the diagnosis of acute myocardial infarction (AMI). Recently, the reliability of the diagnosis of AMI has improved by measurement of the isoenzyme MB of CK (CK-MB), the most sensitive and specific indicator of AMI (5). Few investigators have demonstrated elevated CK-MB levels in patients with skeletal muscle

Table I Participants in the 26 km long jogging race

Subj no	Age (y)	Sex	Years of training	Km run in the month before the race
1	11	♂	4	100
2	18	♀	5	150
3	21	♂	15	50
4	33	♀	6	150
5	37	♂	25	55
6	43	♀	4	145
7	46	♂	7	150
8	50	♀	8	150
9	53	♂	10	100
10	74	♀	15	300

Abbreviations: AMI = acute myocardial infarction, LD = lactate dehydrogenase, ASAT = aspartate aminotransferase, CK = creatine kinase, CK-MB = isoenzyme MB of CK.

Table II Enzyme activities ($\mu\text{kat/l}$) before, just after, 4, 8 and 20 hours after the endurance test (mean and range)

	Before	Just after	4 h after	8 h after	20 h after
CK MB	0 (0-0.1)	0.07 (0-0.25)	0.13* (0-0.37)	0.23** (0-0.47)	0.18* (0-0.33)
Total CK	2.45 (1.05-3.95)	3.90* (1.52-5.93)	6.38* (1.57-14.43)	8.57** (1.57-21.17)	7.38 (1.75-21.00)
ASAT	0.38 (0.27-0.88)	0.57* (0.25-0.97)	0.50** (0.25-0.80)	0.60* (0.27-0.82)	0.57* (0.27-0.93)
LD	5.10 (4.35-6.83)	7.93** (5.6-13.0)	6.78* (4.60-9.75)	6.8** (5.78-9.10)	6.28 (5.55-8.94)

Levels of significance as compared with initial values (Student's *t* test): * $p < 0.05$, ** $p < 0.01$.

Venous blood was sampled at rest within one hour before, just after and 4, 8 and 20 hours after the race. The serum was frozen and stored at -20°C pending analysis which was performed within 72 hours. LD, ASAT and CK were assayed on unhemolysed serum according to the Scandinavian recommendations (9, 10). CK isoenzyme ratios were determined by electrophoresis with fluorescence scanning of the electropherogram (4). The upper reference levels of serum activities based on discriminatory analysis for patients with and without AMI were LD 6.68 $\mu\text{kat/l}$, ASAT 0.67 $\mu\text{kat/l}$, CK 3.33 $\mu\text{kat/l}$ and CK-MB 0.50 $\mu\text{kat/l}$ (5).

RESULTS

Table II shows the mean enzyme activities in the ten volunteers as functions of time. A significant increase was observed in each of the four enzymes. The mean levels of CK-MB and total CK had peak values 8 hours after the race, but the mean values 20 hours after were still significantly higher than the initial values. ASAT activity was significantly raised eight hours after the race. LD activities were significantly raised in all postexercise measurements. The mean hematocrit value was 0.43 before and after the race, suggesting an unchanged plasma volume.

There was no correlation between the increasing

serum enzyme activities and the percentage amount of training. It is obvious from Table I when the discriminatory levels of the four enzymes were used. CK-MB led to no false positive while the three old enzymes yielded 17 false findings.

DISCUSSION

The present investigation confirms the well-known fact that the serum activities of ASAT, LD and CK rise after exercise (3, 6, 11). During the race the pattern of CK-MB was similar to the local pattern. Since trace amounts of CK-MB are found in skeletal muscle (4, 7) and the molecular weight and distribution spaces of CK-MB and total CK are virtually identical, it is likely that the small amount of CK-MB found in serum originates from skeletal muscle, but the heart can of course not be excluded as a partial source.

The fact that the different levels of training in participants did not correlate with the postexercise serum enzyme levels indicates that corresponding results might be seen in untrained persons as well.

None of the participants in the present study showed any clinical signs of AMI after the competition.

Table III Number of participants with serum enzyme activities higher than the discriminatory level

	Before the race (n=10)	Just after (n=10)	4 h after (n=9)	8 h after (n=9)	20 h after (n=7)
CK-MB	0	0	0	0	0
Total CK	2	7	7	7	5
ASAT	1	2	3	3	2
LD	1	5	4	4	3

cal exertion performed. Patients with exercise-induced symptoms of AMI are sometimes added to coronary care units. If these patients do have AMI, determination of the old enzymes lead to false positive results, whereas our findings suggest that serum CK-MB determinations will give false positive results.

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BOOK REVIEW

Assisted Circulation edited by Felix Unger 653 pages
US\$ 110 Springer Verlag Berlin Heidelberg and New
York 1979

This book deals with all types of assisted circulation from the simplest kind of counter pulsation to artificial heart and heart transplantation. Historical background, different technical devices, experimental and clinical results, complications, energy sources, etc. are carefully described in 10 main parts, each part composed of contributions from several international authors.

Part I deals with counter pulsation, external counter pulsation as well as intra aortic balloon pumping. Indications, clinical results and complications are reported. More than 10 years' experience exist now in the field of counter pulsation associated with acute myocardial infarction as well as cardiac surgery.

Part II is the most extensive part of the book, including almost 200 pages about left ventricular assistance devices to be used temporarily postoperatively or for totally intracorporeal implantation and long term assistance (1-2 years) while waiting for heart transplantation. A great deal of the contributions describes different devices and technical solutions that have been tested mostly in the experimental situation and in some cases also in critically ill patients. Of special interest is the section dealing with

combined right and left heart assistance which will be more effective in the treatment of cardiac failure where right ventricular failure of various degrees is common. This is also pointed out by Åke Senn in chapter of the book.

Parts III and IV describe the total artificial heart transplantation where the Stanford Group reports a 3 year survival of 60% after heart transplantation.

Parts V and VI are technical chapters including different aspects of the driving systems for artificial valves.

The concluding parts of the book include current literature during the last 4 years and a chapter: Horizons on the future trends in assistance.

It is impossible to review all the content of this size which includes in much. However, it must be stated that the volume is excellent. It provides interesting and easily richly illustrated (379 figures and 73 tables) recommended for those working in the field of circulation in the coronary care unit or in cardiac surgery and it can also serve as a valuable volume.

Olof Nergst Stockholm

Excessive Sensitivity to the Hyponatremic effect of Chlorpropamide in a Patient with Diabetes mellitus and Anterior Pituitary Insufficiency

Gunnar Aasen and Harald M. M. Frey

From Medical Department B Aker Hospital University of Oslo Oslo Norway

ACT A 67 year old diabetic woman with undiagnosed anterior pituitary insufficiency developed hyponatremic coma within 5 weeks after initiation of chlorpropamide therapy. A provocation test with 500 mg chlorpropamide orally led within five hours to tremor and sopor with high urinary sodium excretion. This rapid development of the hyponatremic syndrome excludes water retention due to ADH as the dominant cause. It is more probable that defects in renal sodium conservation, brought about by the anterior pituitary failure, have been unmasked by chlorpropamide.

Key words: chlorpropamide, hyponatremia, pituitary insufficiency.
Acta Med Scand 208 233 1980

Chlorpropamide induced dilution syndrome has been reported several times since the first case reported in 1970 (13). It was present in 4% of patients with diabetes mellitus treated with chlorpropamide. The mechanism is not fully understood but the evidence suggests enhancement by chlorpropamide of the antidiuretic action of vasopressin in the kidney (16, 17, 18, 30). The ensuing diluting syndrome is indistinguishable from the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) due to other causes, mainly bronchogenic carcinoma, chest infections, (24), different central nervous system disorders and miscellaneous drugs (25). Hyponatremia in SIADH is due to extracellular fluid expansion and secondary sodium loss (5). This report describes severe hyponatremia during treatment with chlorpropamide in a patient with diabetes mellitus who in addition suffered from anterior pituitary insufficiency. Hyponatremia is frequently present in hypopituitarism due to inadequate secretion of both glucocorticoids and mineralocorticoids (3, 6, 7, 11, 14, 15, 21, 27). Lack

of thyroxine (2, 9, 9, 10, 28) and growth hormone are also of importance.

CASE REPORT

A 67 year-old woman with diabetes mellitus since 1970 was placed on chlorpropamide therapy 50 mg daily on July 6, 1977. Within the first weeks of treatment she subsequently developed nausea, irritability, reduced memory and fatigue. On Aug 7, 1977, vomiting occurred and she became soporose. On the next day she was admitted to Medical Department B, Aker Hospital. She appeared well hydrated without edema. A few hours later she became comatose with general convulsions and several plantar reflexes. Cerebral fluid pressure was within normal pressure.

Laboratory examination revealed Hb 11 g/l, hematocrit 0.40, blood urea 5 mmol/l, blood glucose 10 mmol/l, serum sodium 103 mmol/l, serum chloride 93 mmol/l, serum bicarbonate 21.5 mmol/l, serum calcium 1.0 mmol/l, urine osmolality 494 mmol/l. Acid base balance was nearly normal with pH 7.40, pCO₂ 4.1 kPa, bicarbonate 21.5 mmol/l and base deficit 0.

Chlorpropamide and water ingestion were stopped and infusion of mannitol and hypertonic saline was started (Table I). Blood pressure was then 100/60 mmHg during infusion to 130/80. Renin activity 0.1 U/ml, aldosterone concentration (196 pmol/l) in peripheral plasma were within normal limits.

Clinical improvement with returning consciousness ensued paralleling the elevation of serum sodium during saline infusion. Some degree of hyponatremia persisted (mean 130 mmol/l) during the following days with hypotonic urine relative to plasma when on oral water restriction of 750 ml/day and a diabetes diet of 2000 kcal containing approximately 80 mmol/l of sodium daily. Treatment with slow acting insulin 12 IU daily was also initiated. Serum concentration of chlorpropamide was 189 µmol/l on admission, after 3 days 78, on the 4th day 49 and on the 7th day 16.5 µmol/l. To determine her renal diluting capacity an oral water load of 20 ml/kg was given on the 7th day showing insufficiency of maximal urinary dilution (less than 100 mOsm/kg) and delayed excretion (only 40% excreted within 4 hours). Some urinary dilution did however occur and the whole water load was eventually excreted. Continued water loading on the same and on the following 2 days gave confirmatory results (Table II).

Table I Status on admission Aug 8 1977 and response to therapy

Hour	Serum Na (mmol/l)	Osmolality (mOsm/kg)		Urinary Na (mmol/l)	Diuresis (ml/2 h)	Natriuresis (mmol/2 h)	Therapy
		Serum	Urine				
17 20	103	215	494				Mannitol 75 g v (400 m 42 mM 2.9% NaCl (100 m
22	96	225	472	10	755	8	Mannitol 37 g v (400 m
24	103	237	466	8	740	6	210 mM 2.9% NaCl (400 m
07	110	241	538	4	350	1	210 mM 2.9% NaCl (400 m
04	119	244	561	39	250	10	
06	121	247	612	97	90	9	105 mM 2.9% NaCl (400 m
08	125	248	669	60	87	5	
Total					2267 ml/ 12 h	39 mmol/ 12 h	2100 ml H ₂ O 567 mM Na

Further assessment of renal function revealed no abnormality. Serum creatinine was 65 μ mol/l, maximal urinary concentration 948 mOsm/kg, renal isotope scan was normal with normal effective renal plasma flow bilaterally. Glomerular filtration rate was 130 ml/min \times 1.73 m². Adrenal function judged by plasma cortisol indicated normal diurnal values (685 and 435 nmol/l) at that time.

X-ray of the skull and sella turcica was normal. A computerized tomogram revealed some central and cortical brain atrophy. EEG showed organic alterations with a maximum in the left fronto-temporal region which showed improvement at EEG control two weeks later.

To assess the role of chlorpropamide a provocation test with 400 mg orally was performed on Aug 19th. The patient was then on Retard Insulin 12 IU daily and her fasting blood glucose level on that day was 11.4 mmol/l. She was allowed to drink freely. After a few hours she felt ill and became soporose within 5 hours. Serum sodium was then 118 mmol/l. Natriuresis was remarkably high (333 mmol/24 h) (Table III). Serum sodium was 115

mmol/l on the next morning and the condition unchanged. Everted plantar reflexes were present. Infusion with

hypertonic saline (2.9%) was started and consciousness returned gradually after addition of mannitol.

During the next month the patient was in relatively good condition but exhibited reduced memory and persistent fatigue. Hyponatremia with hypertonicity of urine relative to plasma was found on several occasions. A hypotonic and diminished thirst. Urinary sodium loss was apparent although natriuresis was unusually high. On the 4th day after provocation with chlorpropamide (300 mmol/24 h) (Table III). At discharge on Sep 19th serum sodium was 140 mmol/l, natriuresis 730 mmol/24 h, diuresis 1930 ml/24 h. Some urinary loss of sodium was therefore present also when not on chlorpropamide.

The patient's thyroid function was reduced. Thyroxine binding 38 nmol/l, T₄ 1.0 nmol/l and TSH 19. Further investigation of her pituitary function revealed a blunted TSH response to TRH stimulation. (T₄ and fT₄ index/glucagon test showed no significant rise in growth hormone level. Metopirone test indicated low pituitary ACTH reserve. GnRH test showed normal level but no rise in FSH. Urinary 17 KGS were decreased according to Norymberski et al. (10) and urinary free cortisol by the method of Few (12). Urinary free cortisol

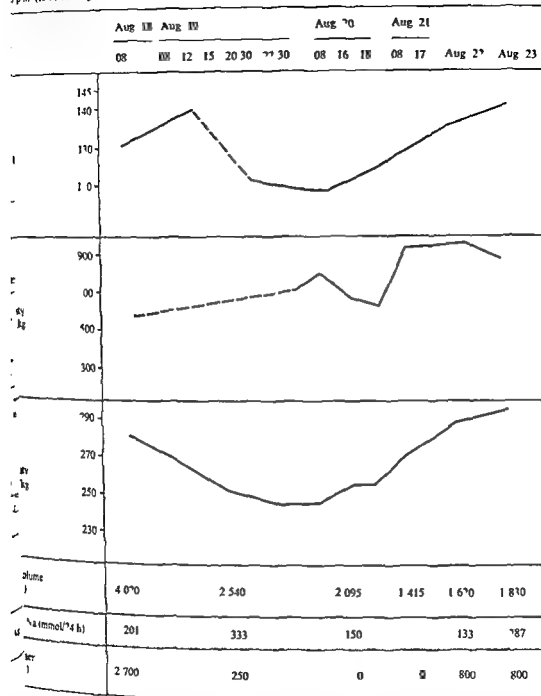
Table II Water load

20 ml/kg on Aug 15 (8 a.m. - 9 a.m.) 3000 ml/24 h on Aug 15, 16 and 17

	Aug 15th						Aug 16th	Aug 17th
	3 a.m.	9 a.m.	10 a.m.	11 a.m.	17:00	1 p.m.		
Serum Na (mmol/l)	131	127	129	130	129	123		126
Serum osmolality (mOsm/kg)	264	262	259	262	255	255		
Urine osmolality (mOsm/kg)								
Urinary Na (mmol/l)		534	364	301	215	180		
Urine volume (ml/h)		135	78	39	27	36		
Urine volume (ml/24 h)		75	105	170	210	200	2770	2910
Urine osmolality (mOsm/kg/24 h)						221	248	291
Body weight (kg)		52.8					51.8	52.1

III Chlorpropam Je provocation

3 pm (15 h) on Aug 19



orbsol were determined as described by Aakvaag
) Plasma ACTH was determined following the
 turer's protocol by a kit from the Radiochemical
 Amenham England. Methods for determination of
 pituitary hormones TSH (25), prolactin (22), LH
 SH (6) and growth hormone (19) have been

published by various authors from the Hormone Labora-
 tory Aker Hospital

The patient thus had partial anterior pituitary insuffi-
 ciency possibly of hypothalamic origin. She was placed
 on thyroxine 0.1 mg daily and was later also given cor-
 tison

Table I Status on admission Aug 8 1977 and response to therapy

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hypertonic saline (2.9%) was started and consciousness returned gradually after addition of mannitol.

During the next month the patient was in relative condition but exhibited reduced memory and peripheral fatigue. Hyponatremia with hyponatremia of unknown origin was found on several occasions although consciousness diminished to zero. Urinary sodium losses were apparent although natriuresis was unusually high. On 4th day after provocation with chlorpropamide (100 mg/24 h) (Table III). At discharge on Sept 19th, sodium was 140 mmol/l, natriuresis 230 mmol/24 h, diuresis 1930 ml/24 h. Some urinary loss of sodium therefore present also when not on chlorpropamide.

The patient's thyroid function was reduced. Thyroxine being 38 nmol/l, T₄ 1.0 nmol/l and TSH 16. Further investigation of her pituitary function revealed blunted TSH response to TRH stimulus (Table I). Lateral glucagon test showed no significant rise in growth hormone level. Metopron test indicated low pituitary ACTH reserve. Glucose test showed normal result but no rise in FSH. Urinary 17 KGS were detected according to Norymberski et al. (10) and urinary cortisol by the method of Few (17). Urinary free cortisol

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Serum osmolality (mOsm/kg)	264	262	259	262	255	255		
Urine osmolality (mOsm/kg)		534	364	301	215	180		
Urinary Na (mmol/l)		135	78	39	77	36		
Urine volume (ml/h)		75	105	120	210	200		
Urine volume (ml/24 h)						3750	2720	2950
Urine osmolality (mOsm/kg/24 h)						221	248	295
Body weight (kg)	57.8						51.8	51.5

Predominant B-Lymphocyte Deficiency in a Case with Lymph Node Disease Resembling Angioimmunoblastic Lymphadenopathy

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ABSTRACT A case with clinical and histological features resembling angioimmunoblastic lymphadenopathy but with a very marked decrease in B-cells instead of T-lymphocytes is presented.

Key words: angioimmunoblastic lymphadenopathy, lymphocyte deficiency.

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Angioimmunoblastic lymphadenopathy was described in 1968 by Lukes and Tindie (5) and angioimmunoblastic lymphadenopathy in 1974 by Frazzera et al (6). These terms may cover a spectrum of abnormal lymph node reactions. Very little is known about the pathogenesis. Graft versus host reaction, autoimmunity, immune B-lymphocyte proliferation and infection with virus, liver extracts, antiepileptics and sulfonamides have been discussed.

MANIFESTATIONS

The most common clinical manifestations are fever, weight loss, rash, pruritus, general lymphadenopathy and hepatosplenomegaly. Laboratory tests are characterized by high B-lymphocyte and low T-lymphocyte numbers, polyclonal hyperglobulinemia, low or absent immunoglobulin classes, lymphopenia, sometimes eosinophilia and Coombs-positive anemia. Histologically, a destruction of normal lymph node structure is seen. There is a proliferation of small vessels, mostly venules with high endothelium, and further a diffuse infiltration with immunoplasma cells and intermediate cell forms and an atypical mononuclear eosinophilic material. Tissue eosinophilia is often seen. The B-cell regions of the nodes are often distinct. There is a histological difference between the cases described by Lukes and Tindie and by Frazzera et al. The latter had a sparse cellularity of the infiltrate, while the former had a highly cellular picture. This variation in histological features may suggest the possibility of a spectrum in cases with the histological diagnoses in

question. However, the cellular type of disease may probably progress to the type with cellular depletion and it seems probable that an abnormal immune response is the common cause, but there may be several subgroups. Some cases are rapidly progressive with or without treatment and die within about 2 years. Others have a better prognosis and respond with remission on either steroid or cytostatic treatment and live for 2-6 years. Some authors however mean that the frequency of severe infections is higher among patients on than off cytostatic treatment (2).

Eosinophilia is regarded prognostically favourable. A number of patients, possibly as many as 10-20%, develop malignant lymphoma (4) but most die in infections. A case with a histological diagnosis of angioimmunoblastic lymphadenopathy (AILD) but predominantly B-lymphocyte deficiency is reported below.

CASE REPORT

A man born in 1903 has a heredity for mild Parkinson. He is a mechanic and has had a slight exposure to asbestos. He has two healthy children.

Hypertension (200/100) was noted in 1972. In the same year a progressive mild Parkinson with pronounced tremor began to develop. He was treated with digoxin, hydrochlorothiazide, levodopa and amantadine. In March 1978 when changing one brand of levodopa to another both with decarboxylase antagonists, he noted pruritus and in November an itching skin rash, chills and lymph node swellings in the right inguinal region.

On admission to the hospital in Dec. 1978 he had massive lymph node swelling in the axillae, supraclavicularly and inguinally and in the neck (Fig. 1). Scintigraphy showed splenomegaly.

ESR was slightly increased (40 mm/h) and platelet counts were constantly low (77-97 $\times 10^9/l$), white cell count was normal (7.9 $\times 10^9/l$) and relative lymphopenia (16%) was constant. Eosinophilia (total eosinophil count about 800 $\times 10^6/l$), high levels of IgG (7.6 g/l) and IgM (9.6 g/l) and a catodal IgG band (3 g/l) were present. Sternal bone marrow smears showed a slight increase in plasma cells. Coagulation status showed an increase in factor VIII. Immunological tests showed IgM antibodies (1/64 titre) against striated muscle and glomeruli and borderline value in acrylfat on test (1/40 titre).

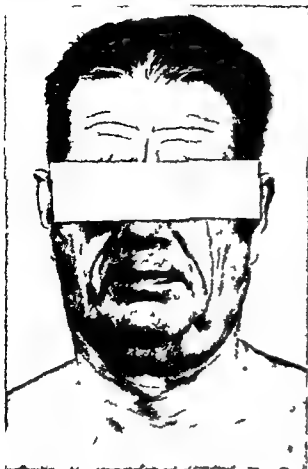


Fig 1 Massive swelling of lymph nodes before treatment with prednisolone

Virus isolations showed herpes simplex infection. He had constant serological titers for cytomegalovirus, rubella and influenza A. No abnormalities were seen in skin sensitivity tests, Coombs test, bacteriophages and bacteriological serology, thyroid function, kidney function, leucocyte phagocytic function and immunoglobulin synthesis.

Histology

The histological picture was that of the cell-rich ALLD described above (Figs 2 and 3). A few small plasma cells were present. There was infiltration of the same type of tissue through the lymph node capsule, the surrounding fatty tissue. The lymph node was interpreted by Henry Rappaport without access to clinical data and he states that the pronounced angiocentric vascular proliferation suggests ALLD. However, characteristic features of ALLD such as the presence of large numbers of plasma cells are lacking. In this case, he has observed a similar non-diagnostic feature which precludes the development of the typical histological picture of ALLD (2).

Therapy

The patient was treated with prednisolone in a dose of 60 mg daily with a successive decrease to daily after 2 months. There was an immediate decrease in eosinophils to almost subnormal values (38 10⁹/l) and 2 weeks of treatment no lymph nodes were palpable. 2 months later IgG was normal (16 g/l) and IgM lower. The immunoglobulin band had disappeared. 4 months later the patient has no signs of the disease and IgM 1.16 g/l.

Lymphocyte studies

Lymphocytes were isolated from heparinized blood and examined by direct immunofluorescence.

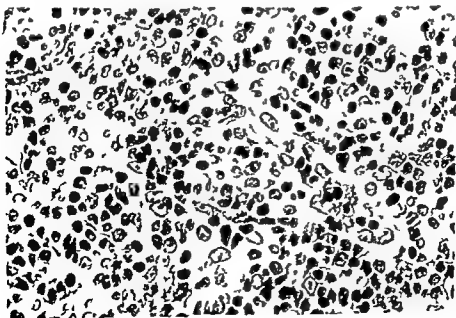


Fig 2 Histological picture of lymph node section showing a blood vessel with high endothelial lining, surrounded by lymphocytes and many large eosinophilic cells (immunoblasts, toxigenic and eosinophilic).



Fig. 3. Histological picture of lymph node demonstrating reticular pattern outlining vascular proliferation. Gridley's reticulin method $\times 95$.

Earlier (8) B lymphocytes were identified by staining with TRITC labelled F(ab') fragments of goat anti-human Fab (Nordic Immunological Laboratories, Tilburg, The Netherlands) or by FITC labelled F(ab') fragments of an anti-CLL serum which was earlier shown to be B-cell specific antigen(s). (1) T lymphocytes identified by the receptors for sheep red blood cells. The percentage of lymphocytes with each marker was determined by examination of 100 cells. The total number of T-lymphocytes per liter blood was then calculated by the total white cell count and differential count of the unadhepantized venous blood. Marker studies were performed before treatment and later when there was definite clinical improvement. The results are given in Table 1. The number of B lymphocytes was very low on both occasions with some tendency to increase after treatment. The

number of T lymphocytes was only slightly reduced. A high proportion of the lymphocytes lacked all markers.

DISCUSSION

The histological lymph node picture of our patient suggested the diagnosis of angioimmunoblastic lymphadenopathy. Clinically the case fits well into this category except for one crucial point, namely, his very low B lymphocyte count. This was confirmed by the concordant finding of a low number of S Ig positive lymphocytes and of lymphocytes expressing a B cell antigen defined by an antiserum against CLL lymphocytes.

Peripheral blood lymphocytes before (I) and after (II) treatment: percentage of lymphocytes and number of cells in whole blood with surface membrane immunoglobulin (S Ig), B cell antigen (r) or sheep red blood cells (SRBC).

	S Ig		B cell antigen		Receptors for SRBC	
	%	$\times 10^9/l$	%	$\times 10^9/l$	%	$\times 10^9/l$
I	0.5	9	nd	nd	57	1069
II	3.5	54	nd	39	77.5	1199
nd duals	1.7	749	12.5	746	80	1593
II	9.75	147-431	9-22	156-403	69-89.5	1261-1780

In almost all reported cases of ALLD there has been a decrease in T cells and an increase in B cells. Neiman et al (6) mean that this depends on a loss of suppressor T cells in a florid immune reaction. Jones et al (3) however report 3 cases with low values of both B and T cells. Our patient is presumably the first case reported with almost normal T cell values and low B cell counts. In addition a high proportion of the lymphocytes lacked B and T cell marker. It is noteworthy that our patient has been able to produce a high number of plasma cells in the marrow and lymphatic tissue and had high levels of IgG and IgM with hardly any circulating B cells. It is also interesting that the clinical and histological picture of the abnormal immune response can be almost the same when it is correlated to a B cell deficiency instead of the more common T cell deficiency and/or B cell increase.

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EDITORIAL

To Treat or not to Treat—Is This the Question?

people would regard it as a truism that all diseases should be treated. The only difficulty may be connected with the definition of the word "disease".

Medical history knows of disease pictures which have lived for a limited time and then disappeared. One of the most remarkable was the so-called status thymico-lymphaticus. It was thought that mainly children suffered from this condition, sometimes it occurred also in adults. The most striking symptom was an enlarged thymus gland. In persons like criminals who were executed or who died suddenly from accidents, the gland was usually surprisingly large. It was generally held that people with this "stigma" were prone to become criminals or die a sudden death from "illness". When the diagnosis was made in childhood, treatment of the thymus was recommended.

The eminent Swedish histologist and endocrinologist Aug. Hammar was able to prove that the size of the gland was much bigger in people who died of a sudden death in contrast to those who suffered from chronic debilitating disease. Chronic disease causes shrinking involution of the gland, is the pathological state? The bigger gland is normal. His findings ended the discussion about the interesting condition. The only disease that was named was the fact that some of the irradiated children developed thyroid carcinoma later in life. In the classical example of the type "noli me tangere" the untouchable condition I think about "treatment" of a non-existing disease may have threatening consequences.

Even if we are quite convinced in our present highly developed medical science that we do not commit any such terrible errors, it may perhaps be appropriate to ransack our own conscience. Many persons with asymptomatic bacteriuria have been unnecessarily treated with chemotherapy and antibiotics. Some of these persons have had severe reactions and some have become seriously ill from the treatment. We live in an age when we cannot give modern potent drugs just in order to feel safe ourselves or know that we have

not missed something. It is a great responsibility to start treatment with many of our modern methods and also to abstain from treatment. For psychological reasons both doctor and patient feel much happier when some treatment is instituted than if nothing is done.

We are now used to find the newspapers full of descriptions and warnings about drugs and this is of course characteristic of our time. It is true, however, that unnecessary medication or operation has been ridiculed for many centuries. Moliere has given us some terrible descriptions, e.g. in *le malade imaginaire*. G. B. Shaw wrote a wonderful comedy about the doctor's dilemma and describes a fashionable surgeon who has detected a new disease. He is unique in being able to make the diagnosis to remove the so-called nuciferous sack and thus enjoy a lucrative practice in this field. It is said that Shaw got this idea from real life. A famous London surgeon was known for his treatment of the so-called Lane's kink in the abdomen that nobody else could find.

The most scathing irony in a description of medical practice is to be found in *Knock ou le triomphe de la medecine* by Jules Romains. It describes the systematic indoctrination of a small city by an unscrupulous young doctor. He teaches the people to become ill and develops an enormous practice by such systematic health care. The whole story is of course a blatant exaggeration but we must admit that many traits in modern medicine are similar in principle. I do not want to shock anybody in particular but many of us have helped to create a feeling of ill health in persons or in groups who do not suffer from anything really serious.

If I take the example renal glucosuria, this is a rare disease and therefore not very important in the population at large. On the other hand, it may cause much trouble for the individual patient. You may read even in modern textbooks that persons who have this anomaly should be carefully observed regarding real diabetes. This is nonsense and is founded on some single observation that a man with

renal glucosuria developed true diabetes. It would of course be strange if all renal glucosurias were immune against such a common disease as diabetes! The underlying mechanisms are entirely different even if glucosuria is the common result.

I now come to my own field of experience. One of the characteristic symptoms in myeloma or macroglobulinemia is the presence of a sharp peak on free electrophoresis of plasma proteins or a sharp band in electrophoresis on solid substrates. Such bands have been called M-components. There has been much discussion regarding the diagnostic importance of such an M-component. It is now clear that only the connection of this finding with other symptoms and signs allows a correct diagnosis to be made. Investigations of large numbers of sera from unselected normal populations have indicated that the presence of an M-component in the large majority of instances does not indicate any serious disease. It is just a biochemical anomaly like renal glucosuria.

The difficulty lies in the fact that it is impossible to state with absolute certainty that this is not the very first symptom of a serious disease. Many doctors may therefore be tempted to use what they would call prophylactic treatment with cytostatic drugs. This is however an absurd idea for several reasons. Only a very small percentage of these persons will ultimately develop myeloma or clinical macroglobulinemia. The number of persons with an ever lasting benign condition is very large and it would be technically impossible to subject all such persons to the supervision that is necessary when cytostatic drugs are being used. The third and most decisive argument is connected with the dangers of long term cytostatic treatment. It is known that acute myelogenous leukemia develops in a small number of patients who have received long term treatment with cytostatics. We may even run the risk that we kill more healthy persons by giving cytostatics—thus causing acute leukemia—than we might possibly postpone the development of myeloma. This is an excellent example when therapy may be more dangerous than the "disease".

There are many similar examples in medicine. When the Allied Forces in World War II were ex-

posed to the risk of yellow fever in Africa, a vaccine was developed. The vaccine however included human serum fortuitously contaminated with the virus of common hepatitis. Many doctors are of the opinion that more healthy persons died from this inoculation than would have been infected and died with yellow fever.

From my personal experience I want to give a final example that shows how treatment of symptoms in a special disease may cause the death of the patient. Acute porphyria needs sedatives. Their acute abdominal symptoms may tempt doctors to make laparotomies in narcosis. It is well known that most sedatives and drugs of general anesthesia cause severe symptoms that even lead to death. The remarkable drop in mortality to almost zero in this condition has been attained by avoiding drugs that are deadly for carriers of the porphyria gene. Similar examples even in modern medicine constitute the modern science of macrogenetics.

Primum est non nocere is an old saying that has to be modified many times when therapy is needed. The old expression "to exorcise the devil with the devil" (to exorcise the devil with the devil) or "to exorcise the devil with the devil" (to exorcise the devil with the devil) is a deadly disease such as acute leukemia it seems permissible to use drugs that may harm themselves be deadly in order to have a chance to obtain some real improvement or even to save the patient's life. The excuse for using drugs that cause severe symptoms of intoxication is of course the edge that there is a fair chance that they may do some good in the long run.

The most controversial chapter in the discussion to treat or not to treat is the use of cytostatics in all patients with solid carcinomas. We know that some of these persons may have a few months even longer of comparative well being if they are not treated, whereas cytostatic drugs usually cause severe side effects during the rest of the patient's life. The therapeutic value is nil in a large percentage of patients. Sometimes life is even shortened by treatment.

To treat or not to treat?—This is a real question.

Jan G. Waldenström

BOOK REVIEWS

Ud of Bulletin Chemical Carcinogenesis vol 1 (1980) Scen Fc Editor P Brookes 104 G 70 Published by The British Council 65 Davies London W1Y 2AA

mbd of the British Medical Bulletin is the fourth in s about chemical carcinogenesis first started by Dr Haddow in 1947 Haddow was one of the leading oncology and he edited the earlier numbers per way In 1958 Kennaway Haddow s predecessor head of the Chester Beatty Institute gave an excellent review of his fundamental work on benzyrenes and importance for the carcinogenic effect of cigarette

present volume also contains many chapters of t for the worker in experimental oncology It is thusic however that hardly more than 4-10 pages problems in human medicine As a matter of fact six pages are devoted to epidemiology some pages fascinating subject of asbestos and other fibres directions with a sick person may otherwise be used cursorily e.g. in the discussion of bacterial dim and human carcinogenesis The explanation on a balance of clinical problems < easy to find the 19 authors only one (the statistician) is an the others are PhD DSc or s m lar

Journal of Biology and Medicine Edited by G Gregorand s 363 pages \$46.00 Academic Press 1979

very important and interesting sensation volume It draws attention to problems that sometimes seem to stem from science fiction At the same time the book is a clinical problem in medical therapy how to reach target when administering a drug One of the most imaginative of medical research in ves Paul Ehrlich wrote about a magic bullet that hit the right target He was also the first to talk specific side chains binding foreign substances to surface Drug Carriers in Biology and Medicine these problems from many viewpoints idea to attach drugs to immunoglobulin molecules & bind specifically to tumor cells is rather recent received great interest at a time when tumor ant very much discussed Other similar drug carriers lectins that interact with carbohydrates in a very way & glycoproteins are also discussed in some Perhaps the most fascinating idea is connected fabrication of small synthetic globules that either substances on the surface or contain such in solution The active drug may be liberated strategic point when transported in the blood Not a few carriers of this kind but also red blood cells & s in activity & sident that most inborn errors of metabolism are

This tendency for the non medically trained to take over medical investigation seems to me really serious Medicine is a subject that should above all treat the problems of sick people Knowledge about such questions of course fundamental for good medical research Two years ago I spent some very stimulating months in Bethesda USA One of my friends there—an eminent investigator—told me with some malice the investigators here are so happy because they think they have found a human model for a murine disease This gives the problem in a nutshell

The volume can hardly be recommended to the clinician but workers in the lab will find excellent and interesting presentations of some of the problems DNA as a target of alkylating carcinogens DNA damage and its repair toxicity of fungal products are some examples Ethyl nitrosourea and brain tumors in the rat is a fascinating story illustrated by experiments on cells in tissue culture

Let us hope that the fifth number in this important series will be more clinically oriented

Jan G Waldenström

caused by a lack of some specific enzyme One example is the specific type of hereditary hypogammaglobulinemia that seems to be caused by a deficiency of adenosine deaminase This enzyme prevents the accumulation of adenosine a substance that is obviously toxic for immunocytes that produce immunoglobulin Transfusions of normal erythrocytes have exerted a favourable influence probably through the content of the lacking enzyme Other similar metabolic disturbances may be attacked in the same way These methods of administration seem to be more promising than intravenous injection of enzyme that is rapidly destroyed or excreted It is possible that the same principle may be used also in the treatment of Gaucher's and Fabry's diseases

Another possible way of attacking such problems would be to transplant some organ that contains cells producing the lacking factors Perhaps the first idea of this kind was tested by the transplantation of normal spleens to dogs suffering from canine hemophilia These experiments were not very successful The idea has also been followed up in human medicine by the transplantation of normal kidney to a patient with Fabry's disease These patients finally die from kidney disease and it is therefore indicated with kidney transplantation In one patient the procedure seemed to give promising results also as regards the metabolic defect and not only by correcting the renal shutdown Spleen transplants have also been tried in Gaucher's disease

Even if such transplantation experiments obviously are

connected with many complications. It seems as if transplantation of cells carrying active substances and surviving in the recipient with continued production would be more practical. Interesting experiments have been made by infusion of normal liver cells into animals suffering from metabolic diseases of the liver. Survival of the transplant is of course the problem.

Already in 1936 autotransplantation of parathyroid glands was performed after total thyroidectomy. Such examples have been performed on a large scale and there is no question about the fact that a take occurs also functionally.

The most exciting type of experiment with this technique has been performed by pancreatic surgeons. It seems as if also allografts may survive if islet cells are injected into the portal vein. The liver is obviously an excellent soil for the culture of islet cells. Such experi-

ments work surprisingly well in rats. In man, the techniques of islet cell transplantation have been tried in diabetics who were on immunosuppression because of previous kidney transplantation. Perhaps the most promising situation could be autotransplantation of islets prepared from the surgically removed organ in a patient with chronic pancreatitis and severe pain. The experiment is still very limited but the idea is promising.

Japanese authors have been much interested in using artificial membranes that may be biodegradable and release their content of active substances. It is possible that such plans are only the products of free speculation. On the other hand it seems appropriate to follow such developments and the contributors to this volume are eminently competent to describe the present day state of affairs.

Jan G. Waldenström

Atlas of Human Hemopoietic Development By E. Kelemen, W. Calvo and T. M. Fliedner. 266 pages. US\$202.40. DM 368.— Springer Verlag, Berlin, Heidelberg and New York, 1979.

As stated by Marcel Bessis in the foreword, the present book makes us realise once more that morphology is the science of structure and shape and that its aim is not to collect pictures but to understand them. The authors have used the 22 first pages for an introductory text part about the extra-embryonic and fetal hemopoiesis. Here the data, based on studies of 190 human embryos and fetuses, are summarized in nine tables and five figure curves which show the quantitative change in the cellular composition of the hemopoietic organs during embryonic and fetal development.

The most impressive part of this book is certainly not the text and tables but the photographs. They are of the highest quality both in black and white and in colour. An artist would envy the picture of circulating primitive erythroblasts in the 7–8 week old embryo. The use of various magnifications and also electron microscopic photographs enhances the impression of the dynamic progression of events. Each photographic plate is explained

on the opposite page. Unfortunately, however, the explanatory symbols are very rarely used on the photographs. From the artistic point of view this might be an advantage but for the reader it is a time-consuming task to find the right cell among 20–30 when indicated only by a small dot located at the center left at lower third of the page.

The wonderful photographs are also poorly used in the introductory text part. Although this part is written in the same way as the plates, i.e. diffuse hemopoiesis, intravascular hemopoietic cells and organ bound hemopoiesis, referral to the plates is only rarely made. Referral at all is made to the electron microscopic plates. The dynamics of events at the subcellular level is therefore poorly clarified to the reader.

These limitations should not blur the view that this book is probably unsurpassed for those who want to see the morphology of hemopoiesis during embryonic and fetal development. It is a book for the specialists, the researchers and the institutions (hematologic, morphologic, pediatric and cytologic). The price will probably limit its spread.

Gosta Gahrton, Huddinge, Sweden

Smoking Habits in the Glostrup Population of Men and Women, Born in 1914

Implications for Health Evaluated from Ten Year Mortality Incidence of Cardiovascular Manifestations and Pulmonary Function 1964-1974

Marianne Schroll

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ABSTRACT Of a total population of 514 men and women born in 1914, from seven municipalities in Copenhagen County 87% were examined in 1964 and 114. Smoking habits (inhalation kind of tobacco, amount, duration of smoking and changes over time) have been described in this age-specific, representative population. The health implications were assessed. Tobacco consumption was in this population the most important risk factor for overall mortality, cardiovascular manifestations, peripheral arterial disease, decline in pulmonary function and occurrence of ulcer. The results are consistent with prospective epidemiological studies. The relationship of death, of fatal and nonfatal cardiovascular manifestations and of decline in pulmonary function increased gradually with the amount of tobacco inhaled. Inhalation roughly doubled the risk. The risk for pipe/cigar smokers was less than for cigarette smokers. Ex smokers reduced their risk about 60%. The risk associated with smoking was independent of other factors. The excess risk was about the same in women as in men, but the community problem of smoking related disease was most pronounced in men, among whom smoking habits are more widespread and morbidity higher. The results from the 1914 population study show that almost one third of all deaths and heart attacks in middle-aged Danes might be avoided, if 15-year-olds would have given up smoking.

ten year mortality and incidence of smoking related diseases in an age specific general population of men and women according to duration of smoking, inhalation amount and kind of tobacco smoked.

STUDY POPULATION AND METHODS

Population

The 1914 population in Glostrup comprises the 514 men and 461 women (the total population of 50-year-olds) who in 1964 were living in seven municipalities around the Copenhagen County Hospital in Glostrup. 802 were examined at entry (14). In 1974 the 890 survivors were reinvited and 666 participated in the reexamination.

Procedure

A description of the selection and representativity of the population and the design of the study are given elsewhere (14, 28). The surveys of 50- and 60-year-olds included questionnaires, fasting blood and urine specimens, resting and exercise ECGs, pulmonary function tests, X-rays and a social-psychiatric section. When available WHO standardized questionnaires were used (27). Questions about smoking habits are Danish translations of the questionnaire about smoking habits (27). Only one interviewer posed the questions to all participants in a single survey (L. Hagerup in 1964, M. Schroll in 1974). The answers were transformed into variables describing different aspects of the smoking habits: 1) Tobacco consumption (38): 1 cigarette=1 g, 1 cigarillo=3 g, 1 cigar=5 g, never smokers, ex smokers, smokers of 1-14, 15-24 or more than 25 g/day. 2) Tobacco consumption (g/day \times no. of years of smoking). Lifetime tobacco consumption (kg). 3) Smoking habits: never smokers, ex smokers, cigarette smokers, smokers of pipe, cigars and cigarillos, mixed consumption.

Abbreviations: CVD=cardiovascular disease, AMI=acute myocardial infarction, FEV₁=forced expiratory volume in 1 sec.

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and bronchitis, cardiovascular diseases, mortality, active studies, respiratory function tests, smoking. Med Scand 1980; 245: 245-256.

Smoking is a significant, probably causal risk factor for total mortality and cardiopulmonary diseases in Western industrialized countries, as shown in several prospective epidemiological surveys (26). The 1914 population study gives a picture of the

Table I Smoking habits in the 1914 population in Glostrup at the age of 50 in 1964

	Males		Females		Total	
	N	%	N	%	N	%
Present smokers	340	78	217	59	557	69
Inhalers	247	57	115	31	362	45
Never smokers	42	10	128	35	170	21
Ex smokers	54	12	21	6	75	9
Consumption on per day (g)						
1-14	136	31	151	41	287	36
15-24	175	29	52	14	177	22
>25	76	17	11	3	87	11
Unknown	3	1	3	1	6	1

control of inhalers on nonsmokers and non-inhaling smokers inhalers. 5) Ex smokers for more than 10 years (quit smoking before 1964 and did not resume) for less than 10 years (quit smoking between 1964 and 1974 but did not smoke for one year before the survey).

The implications of smoking for health have been evaluated by relating habits to ten year mortality, ten year incidence of fatal and nonfatal cardiovascular diseases (CVD) and pulmonary function.

Copies of death certificates of participants who had died between 50 and 60 years of age were obtained from the National Health Service. The causes of death were verified from discharge records of hospital cases. Autopsy was performed in 68% of the 85 deaths. Causes of death were registered according to the international classification (ICD) (37). Information about cardiovascular manifestations (CVD) at the age of 50-60 were derived from death certificates, discharge records, the Copenhagen Heart Register and the survey in 1974. The ten year incidence of

CVD includes fatal and nonfatal cases of acute myocardial infarction (AMI) (ICD 410) in the heart, death due to aortic (ICD 390-394) or aortic heart failure (ICD 411-419), cerebrovascular attacks (ICD 430-434), peripheral arterial disease when verified by angiography (ICD 440). Fatal cases verified by autopsy and other cases in whom two of three clinical criteria (typical serial ECG and enzyme changes) pointed to the diagnosis were regarded as cases of myocardial infarction. Attacks with focal neurologic symptoms of less than 24 hours duration and of more than 24 hours duration were regarded as cerebrovascular manifestations. For one woman had had a CVD before 1964. This subgroup in the multivariate CVD analyses using the BMRC questionnaire on bronchitis and pulmonary function tests have been described thoroughly (forced expiratory volume in 1 sec (FEV₁) was measured by a Godart expirograph. The change in pulmonary function between the ages of 50 and 60 years (the slope of the connecting 50 and 60 year values) was compared with differences in FEV₁ entry by division with the value

$$\frac{FEV_{60} - FEV_{50}}{FEV_{50} + FEV_{60}}$$

Smoking habits have been related to other variables: constitutional factors, social background, living habits and health indicators (9).

The information was checked for missing and unreliable values and analyzed in the SCIBAS system (10). Univariate analyses: nonparametric statistical tests used: χ^2 , Wilcoxon, Mann-Whitney, Kruskal-Wallis, the Spearman rank correlations. To elucidate the smoking independent of other risk factors in the analyses were applied. With time between the last survey in 1964 and death or first cardiovascular manifestation as end point, the Cox regression model (11) was

Table II Smoking habits at the age of 60 in 1974 in the 1914 population in Glostrup

	Never smokers	Ex smokers	Consumption on per day (g)			Inhalers
			1-14	15-24	>25	
Men (n=360)						
Never smokers	38					
Ex smokers		79				31
Cigarette smokers			36	48	11	85
Pipe and cigar smokers			55	40	22	44
Mixed consumption			12	11	8	25
Total	38	79	103	99	41	185
Women (n=306)						
Never smokers	120					
Ex smokers		26				6
Cigarette smokers			80	70	3	16
Pipe and cigar smokers			41	11	4	
Mixed consumption				1		
Total	120	26	121	32	7	83

III Changes in smoking habits between the ages of 50 and 60 in 670 men and women examined in 1974

	Age 60				
	Never smokers	Ex smokers	1-14 g/d	15-24 g/d	>25 g/d
<i>n</i> (33%)					
smokers	76	7	2	0	0
ex smokers	5	33	7	2	1
consumption per day (g)					
1-14	4	18	52	30	3
15-24	1	12	27	44	14
>25	1	7	7	16	21
<i>n</i> (85%)					
smokers	97	2	5	1	0
ex smokers	6	9	2	1	0
consumption per day (g)					
1-14	11	12	78	10	2
15-24	1	2	19	15	3
>25	0	0	3	3	3

lung function at the age of 60 as end point analyses were applied (36). In the multivariate analysis on the coefficient with its standard deviation gives a test statistic (the U-obs value) 1.96 at the 1% level. The strength of the association in smoking habits at entry and ten year mortality is expressed as the "relative risk".

Incidence (mortality) among smokers
by category of smokers

Incidence (mortality) in nonsmokers

The "attributable or excess risk" is incidence among smokers—incidence among nonsmokers.

Relating the attributable risk to the distribution of smoking habits gives the population attributable risk. Attributable risk \times prevalence of a certain category in the population.

RESULTS

Smoking habits

Table I shows the smoking habits in the 1914 population at entry in 1964 at the age of 50. The sex

IV Ten year mortality related to smoking habits at the age of 50 at entry

	<i>N</i>	Deaths	Absolute risk per 1000	Relative risk	Attributable risk per 1000	Population attributable risk	
						<i>N</i>	%
smokers	47	2	48 (6-167)	1.00	-	48	9.4
ex smokers	54	4	74 (21-179)				
consumption per day (g)							
1-14	136	13	96 (50-154)				
15-24	125	15	120 (66-184)	1.46	38	108	21.2
>25	76	17	224 (136-334)	2.73	142	108	21.2
Total	433	51	117 (94-167)				30.6
nonsmokers	128	4	32 (8-77)	1.00	-	18	9.5
ex smokers	21	1	48 (1-238)				
consumption per day (g)							
1-14	151	8	53 (23-103)				
15-24	52	4	77 (21-185)	1.79	34	15	7.9
>25	11	2	182 (23-518)	4.23	139	15	7.9
Total	363	19	52 (29-73)				17.4

Table V Ten year mortality related to inhalation at the age of 50 at entry

	N	Deaths	Absolute risk per 1000	Relative risk	Attributable risk	Population attributable risk	
						A	=
Men							
Noninhalers	196	13	66 (35-109)	1.00	-	-	
Inhalers	240	38	158 (113-207)	2.39	92	"	41
Total	436	51	117 (94-162)				43
Women							
Noninhalers	250	11	44 (22-78)	1.00	-		
Inhalers	116	8	69 (29-128)	1.57	25	2.9	15
Total	366	19	52 (29-73)				15

difference was pronounced 35% of females and 10% of males had never smoked 70% of the female smokers had a moderate consumption compared to 40% of the male smokers 48% of female smokers and 76% of male smokers were inhalers

Table II gives the corresponding information about smoking habits in 1974 at the age of 60. There was a tendency towards a reduction of consumption as the percentage of ex smokers had doubled thereby diminishing the other smoking categories. Table II combines information on amount with the type of tobacco smoked. In this elderly Danish population a strikingly high proportion (48%) of the men smoked a pipe, cigars or cheroots. The distribution of pipe/cheroot smokers over moderate, medium and high tobacco consump-

tion was 47, 34 and 19% which does not differ much from the distribution of cigarette smokers roughly 10, 20 and more than 20 a day 36, 41 and 15%. No women but 25% of the men were smokers (12 g/day on average). Only 1% of women and 5% of the men smoked cigars. Cheroots were preferred by 25% of the men and 18% of the women (6/day). At 60 years 35% of the men and 34% of the women smoked cigarettes (22 and 11/day respectively). Of cigarette smoking men 87% were inhalers compared to 38% of the pipe/cheroot smokers. This ratio was the same among women.

Table III gives the individual changes in smoking habits between the ages of 50 and 60 but it also points to some methodological problems.

Table VI Ten year mortality related to kind of tobacco smoked at the age of 50 at entry

						Populat on attributable risk	
	N	Deaths	Absolute risk per 1000	Relative risk	Attr but able risk per 1000	A	C
Men							
Never smokers	42	2	48 (6-162)	1.00		-	
Ex smokers	54	4	74 (21-179)				
Pipe and cigar smokers	152	14	92 (52-157)	1.46	29	4.4	8.6
Cigarette smokers	188	31	163 (114-224)	2.67	107	19.7	37.6
Total	436	51	117 (94-162)				46.2
Women							
Never smokers	128	4	31 (8-77)	1.00	-	-	
Ex smokers	21	1	48 (11-238)				
Cigarillos	67	3	45 (9-125)	1.32	11	0.7	3.7
Cigarettes	140	11	73 (77-128)	2.15	39	5.9	31.1
Total	366	19	52 (29-73)				34.8

II Cardiovascular manifestations related to smoking habits at the age of 50 at entry

	N	CVD	Absolute risk per 1000	Relative risk	Attributable risk per 1000	Population attributable risk		
						N	%	p
Smokers	41	7	48 (6-167)	1.00	-	16	3.3	0.07
Less	14	7	130 (54-249)					
ption per day (g)								
	136	12	88 (45-145)					
	174	13	104 (69-179)					
	76	15	197 (115-305)	2.16	108	87	16.7	
	431	49	112 (97-156)				20.0	
Smokers	178	0	0 (0.0-78)	1.00	-	46	25.6	n.s.
(men)	21	0	0 (0.0-161)					
ption per day (g)								
	151	8	53 (47-136)					
	57	6	115 (44-234)	4.26	88.0	46	25.6	
	11	4	364 (109-697)	13.48	337.0	37	70.6	
	363	18	50 (7-70)				46.2	

the information takes the form of answers to standardized interviews. It depends on a judgement as to whether it is surprising to find a transition between adjacent groups, but 18 smokers at the age of 50 ought to be described as never smokers at the age

of 40. Of 11 men and 9 women classified as ex-smokers at the age of 50, 10 took up smoking again before the age of 60. Another 37 men and 14 women gave up smoking. Once established smoking habits are difficult to change, making it possible to estimate individual life consumption. This averaged 10 g in men and 75 g in women. The pronounced difference was due to the higher prevalence of

non-smokers among the women and a later start. Most of the men started to smoke around the age of 20-25% already between 10 and 14. Half of the women started after the age of 45, only 25% before the Second World War and only 10% before the age of 20.

Implications for Health

Ten year mortality (all causes) among men and women according to smoking habits at the age of 50 (Table IV)

The total ten year mortality was 11.7% among men and 5.2% among women. Only 69% of the 1914

IV Ten year incidence of cardiovascular manifestations in relation to inhalation at the age of 50 at entry

	N	CVD	Absolute risk per 1000	Relative risk	Attributable risk per 1000	Population attributable risk		
						N	%	p
Smokers	196	17	87 (50-133)	1.00	16	11.0	2.4	0.007
	240	32	133 (89-176)	1.53				
	436	49	112 (97-156)				2.4	
Non-smokers	250	6	32 (9-57)	1.00	54	6.3	34.8	n.s.
	116	10	86 (41-148)	2.69				
	366	18	49 (27-70)				34.8	

Table IX Ten year incidence of cardiovascular manifestations in relation to kind of tobacco, age of 50 at entry

	N	CVD	Absolute risk per 1000	Relative risk	Attribut- able risk per 1000	Population- attributable r	
						N	%
Men							
Never smokers	42	2	48 (6-162)	1.00	-	-	-
Ex smokers	54	7	130 (54-249)				
Pipe and cigar smokers	152	16	105 (62-168)				
Cigarettes	188	24	128 (83-182)	1.36	34	6.4	13
Total	436	49	112 (92-136)				16
Women							
Never smokers	128	0	0 (0-28)	-	-	-	-
Ex smokers	21	0	0 (0-161)	-	-	-	-
Cigarillos	67	5	75 (25-166)	?	75	5.0	7
Cigarettes	150	13	87 (47-144)	?	87	13.0	12
Total	366	18	49 (27-70)				100

population were smokers but 84% of the deaths occurred among smokers a significant overmortality ($p < 0.03$). The relative risk increased steadily with increasing amounts of tobacco smoked per day by a factor that was not smaller for women. With small numbers in each class confidence limits of absolute risk overlapped and relative risk has been calculated in relation to a combined group of non- and moderate smokers. Attributable risk measures here the excess mortality of >15 g tobacco per day among smokers. Population attributable risk gives a theoretical maximum value for the percentage of ten year mortality in the population under observation that would have been avoided if the 50-year olds had stopped smoking (15 of 51 deaths in men).

Table V shows the same calculations for inhalation a habit which significantly doubled the mortality among men, even though the group of

noninhalers included quite a large number who consumed a considerable amount of tobacco per day.

Table VI shows that the risk for ex smokers was greater than that for ex smokers the risk for cigarette smokers doubled it.

Causes of death were mainly myocardial infarctions (35%) and cardiovascular disease. No significant overmortality from myocardial infarctions among smokers in this population in ten years.

Ten year incidence of fatal and nonfatal cardiovascular manifestations in men and women to smoking habits at the age of 50

Table VII shows that 87% of the cardiovascular manifestations occurred in the 69% of the population who smoked at the age of 50. The relative risk of smoking was especially

Table X Lifetime tobacco consumption related to morning cough/phlegm at the age of 60 population in Glostrup

	Men (N=360)		Women (N=306)		Total (N=666)		
	Consumption (kg)	S.D.	Consumption (kg)	S.D.	Consumption (kg)	S.D.	P
No symptoms	206	194	59	100	133	171	<
Symptoms	295	226	123	109	233	209	
All	238	211	75	105	163	189	

XI Pulmonary function and fall in pulmonary function at the age of 50-60 in relation to smoking at the age of 60 in the 1914 population in Glostrup

	FEV (l)				FEV (50-60) 00 FFV (50+60)			
	360 men***		306 women*		360 men* *		306 women	
	\bar{x}	S D	\bar{x}	S D	\bar{x}	S D	\bar{x}	S D
nokers	3.03	0.5	2.10	0.4	9.9	9.9	7.7	13.5
ers before 1964	2.86	0.5	2.04	0.4	10.3	10.7	4.4	19.5
ers after 1964	2.74	0.6	2.05	0.5	15.4	16.9	8.7	7.6
ption per day (g)								
	2.80	0.5	1.94	0.5	10.7	17.3	14.2	16.4
	2.58	0.6	1.93	0.4	16.4	15.3	17.1	11.5
	2.74	0.6	1.99	0.3	8.9	15.5	9.9	8.4
par smokers	2.84	0.5	2.03	0.4	11.0	13.7	11.7	13.7

** rank correlation R value between FEV and kg tobacco/life was 0.16
p < 0.001

only female smokers had heart attacks at the decade. As the incidence of CVD was high among male ex-smokers there was no excess risk for male smokers.

Table VIII shows that inhalation was of importance to the increased incidence of CVD among smokers. Only 5 of 31 myocardial infarctions among men occurred in non-smokers ($p < 0.004$). Table IX it will be seen that pipe/smoker had a lower incidence than cigarette smokers when the amount of tobacco smoked in the pipes was almost the same (Table III).

Smoking and atherosclerosis

The prevalence and incidence of angina pectoris among the Rose questionnaire were not re-

lated to smoking habits in this population at entry. Neither was there any strong or consistent relationship between ECG changes and smoking habits. Smoking was a highly significant risk factor for peripheral arteriosclerosis disease. In a separate report (31) it is shown that the correlation between lifetime tobacco consumption and the index of ankle/arm blood pressure was $R = 0.77$.

Pulmonary symptoms as a function in relation to smoking habits

Table X shows the highly significant association between morning cough/phlegm and lifetime tobacco consumption (age 60). From Table XI it will be seen that pulmonary function (FEV) and change in FEV with age (or over time) were more favourable among non-smokers than smokers. An interesting

XII Multivariate analysis (Cox regression model) of the relationship between time of death (to examination in 1974) and six baseline variables measured at the age of 50 in 1964

Variables	Coefficients	S.D.	U-obs	Level of significance
Smoking habits				
non-smokers				
consumption per day (g)	0.6161	0.6961	0.8850	n.s.
4	0.7690	0.5666	1.3571	n.s.
5	1.1549	0.5832	1.9803	$p < 0.05$
Heart rate	1.6555	0.609	2.7458	$p < 0.01$
	0.0240	0.0091	2.6270	$p < 0.01$
Glucose and high insulin levels	-0.2553	0.4057	0.692	n.s.
Glucose and high insulin levels	1.0137	0.4848	2.0909	$p < 0.05$
	0.8370	0.2731	3.0645	$p < 0.01$
	0.0760	0.0223	-3.4079	$p < 0.01$
	1.1553	0.3533	3.2704	$p < 0.01$

Table XIII Multivariate analysis (Cox regression model) of the relationship between time of cardiovascular manifestation (or follow up examination in 1974) and seven baseline variables measured at the age of 50 in 1964

All variables were available in 790 persons after exclusion of CVD cases before the age of 40 or cardiovascular manifestation before the age of 60

Baseline variables	Coefficients	S D	U-obs	Level of significance
Diastolic BP (mmHg)	0.0327	0.0106	3.0955	$p < 0.0001$
Smoking habits				$p < 0.0001$
Ex smokers	2.7216	1.0327	2.1903	
Consumption per day (g)				
1-14	2.6191	1.0327	2.5162	
15-24	3.0888	1.0426	2.9625	
>25	3.3994	1.0590	3.2100	
Ischaemia (age 50)	0.7227	0.2969	2.4343	$p < 0.05$
Physical activity at work				$p < 0.05$
Moderate active	-0.6722	0.3158	-2.1287	
Active	-1.0521	0.3963	-2.6042	
Heavy work	-0.6171	0.4753	-1.0726	
No. of rooms at home	0.2091	0.1099	1.9023	$p < 0.05$
Relative weight (% of Natvig's tables)	0.0112	0.0075	1.4827	n.s.
Male sex	0.2927	0.3125	0.9367	n.s.

difference was observed in FEV_{10} in ex smokers for more and less than ten years respectively. The FEV_1 of pipe/cherry smokers was intermediate.

The relationship between smoking and other variables—the independent risk of smoking

Smoking habits were significantly related to other variables measured at the ages of 50 and 60 years (29). As these relations might modify the association between smoking habits and death/CVD/ FEV_1 , they have been included in multivariate analyses of

the independent risk of smoking. They were: marital status, number of rooms in apartment, consumption of alcohol or tea, leisure activity and max. aerobic power, pressure, resting heart rate, overweight, diastolic blood pressure and pulmonary function at entry.

Table XII gives the relationship to FEV_1 of different smoking groups, controlled for the mentioned baseline variables (Cox regression). Only the six variables of significant importance in the risk factor model are given in the table.

Table XIV Logistic analysis (Duncan-Walker) of the relationship between low FEV_1 /height (the left tail of the distribution) and six variables measured at the age of 60 and univariate associated variables

	Coefficients	S D	U-obs	Level of significance
Constant	-0.90001	0.7001	-1.2856	n.s.
Male sex	-2.2607	0.2415	-9.3611	$p < 0.0001$
Cough in the morning	1.1210	0.2413	4.6461	$p < 0.001$
Ex smokers/nonsmokers	0.2091	0.3443	0.6072	n.s.
Cigarette smokers/nonsmokers	0.7133	0.2614	2.7282	$p < 0.01$
Pipe and cherry/nonsmokers	0.1571	0.2859	0.5496	n.s.
Mixed consumption/nonsmokers	0.2670	0.5928	0.4503	n.s.
Social status class				
5/1-4	0.4867	0.6510	0.7476	n.s.
6/1-4	1.2466	0.5642	2.2097	$p < 0.05$
7/1-4	0.9815	0.5678	1.7284	n.s.
8/1-4	1.2335	0.5768	2.1186	$p < 0.05$
No. of rooms in apartment	-0.1117	0.0763	-1.4649	n.s.
Average no. of drinks/day	-0.0079	0.0498	-0.1593	n.s.

cent for pipe/cheroot smokers was similar to ex smokers and moderate smokers (0.81). Table XIII gives accordingly the risk factors for a ten year incidence of CVD. The coefficients are higher for daily use of tobacco than for the variable indicating the independent incidence of smoking as a cardiovascular risk factor. The coefficient for pipe/cheroot smokers was 0.9 in a similar analysis where only amount of tobacco smoked was exchanged with the kind of tobacco smoked as baseline. Other confounding variables related to smoking habits at entry did not independently influence the time of CVD from baseline information that is there was no statistically significant difference between their coefficients and they are therefore not given in the table. Table XIV shows that pulmonary function at the 1964 was directly related to sex, symptoms of chronic smoking habits and social status according to Svastog classification (28). The risk for low FEV₁/height was more pronounced in men due to body build, in coughers, in cigarette smokers and in people with a low social status and few rooms in the apartment. Alcohol intake was unrelated.

DISCUSSION

A cohort of Danes born in 1914, nine out of ten men and only two out of three women had been smokers. The women had also started to smoke earlier in life than the men. The mean tobacco consumption was moderate. A considerable part of the smokers were pipe/cheroot smokers. Two thirds of the smokers were inhalers.

The 1914 population is an age specific, total population from a defined geographic area. It comprises 17% of the 1914 birth cohort in Denmark. At the selection in 1964 it was representative of the 1914 cohort in sex, social and occupational distribution (14). Ten year mortality and incidence of CVD were also the same as in the 1914 birth cohort (28).

Of the persons originally invited, 13% never appeared at the health examinations. Non participants for whom baseline data were obtained at the age of 60 differed from participants examined in smoking habits (fewer men were non smokers), pulmonary symptoms (more men had a chronic cough) and pulmonary function (lower FEV₁ values in men as well as women) ($p < 0.05$). The differences between smoking and tobacco-related

diseases derived from examination of the participants will probably not be less pronounced in the non participants.

The age specificity (all participants born in 1914) facilitated the interpretation of results because the incidence of diseases in the heart and lungs is age specific and to a different degree in the two sexes. Smoking habits also differ between birth cohorts due to differences in individual influences over time (secularization). The smoking habits described in the 1914 population accordingly represent smoking habits of Danes in that year.

Studies on tobacco consumption of Danes have been published before (3, 16, 17, 21, 22, 23). The figures in Table III in the present paper are almost identical with those concerning 50–64 year old Danes in a Gallup market analysis in 1975 (21). The influence of the neighbouring capital Copenhagen might be mirrored in a slightly higher frequency (52%) of female smokers in Glostrup than in the country as a whole (44%).

The smoking habits in the 1914 population seem quite unchanged since they were established as seen when compared with the Danish National Morbidity Survey in 1952–54 (16, 17, 23), i.e. when the 1914 population was 40 years old. Their smoking habits in 1974 (Table III) are very similar to those of 30–39 year olds in the morbidity survey but differ from 50–59 year-olds at that time. Stated age of participants when starting to smoke was also in agreement with our findings: most men born 1911–14 started to smoke before the Second World War compared to only one third of the women. The 1914 population differs from younger generations of Danes (21, 22). The tendency during the century has gone towards fewer smokers, more female smokers and more inhaling cigarette smokers.

Differences in smoking habits were also observed when the Glostrup population was compared with age comparable foreign populations (e.g. Swedes, Americans). Only 56% of the 50-year old men from the 1913 population in Gothenburg were smokers (35) though as many as in Glostrup were cigarette smokers (47%) since only 9% smoked pipe/cigars. The proportion of male smokers in the USA is the same as in Denmark but individual consumption is higher and the majority are cigarette smokers. More Danish than American women of this age were smokers and their use of cheroots is a national characteristic (21, 33). The differences regarding the year of birth and na-

trationality must be kept in mind when the results from the multivariate analyses of smoking and related diseases are compared between populations.

Overmortality among middle aged male smokers is known from several prospective studies (9, 10, 11, 26, 35). The results of the ten year mortality study in Glostrup are consistent with these findings showing that Danish male smokers are at increased risk like smokers of other nationalities. The figures in Table VI do suggest a similar relative risk in female smokers although the numbers here were not statistically significant. This is important for the individual female smoker. The community problem was less pronounced in females as only 3 deaths in 366 women could be attributed to overmortality among smoking women compared to 15 excess deaths in 436 men.

The proportion of pipe/cheroot smokers was larger than in most studies making it possible to estimate the absolute risk for male non cigarette smokers. This was only a little higher than that for never smokers and ex smokers. The relative risk compared to never smokers was 1.9. In the 1913 population in Gothenburg it was higher 2.8 and similar to that of cigarette smokers (35). Differences in inhalation habits might explain the discrepancy.

The strong association (high relative risk) of smoking with mortality makes it probable that smoking is a causal risk factor (26). This possibility was strengthened by the gradually increasing risk with increasing amount of tobacco smoked and the independency of other risk factors. The effect of smoking on mortality was even increased when allowance was made for the influence of overweight and hypertension. The hypothesis that the association between smoking and death was secondary to some underlying characteristic (4) was tested indirectly with the same result as in some other studies (9, 10). None of the confounding variables were independently associated with ten year mortality making the hypothesis improbable. This implies that the harmful effect of smoking is due to the smoke. The psycho social risk factors of becoming a smoker will be the subject of another analysis of the 1914 investigation.

The mortality of the ex smokers was lower than that of smokers. As some of the registered ex smokers may have taken up smoking again after the baseline survey (Table III) and as some had been ex smokers for only a short time the possibility remains that persistent ex smokers may have the

same mortality after some years as never smokers (11, 15).

The effect of intervention against smoking is the last and important argument in the discussion of causality—has not been examined in the 1914 population in Glostrup as the population was not touched between the surveys. The purpose of descriptive and analytic Monofactor interventional studies against smoking in controlled clinical trials has effected successfully after myocardial infarction (18). Primary prevention trials are under way. On average 25% of the participants remain smokers a year after the intervention. The effect on mortality of this has not yet been evaluated.

Smoking is known to be the most important causal factor for the increase in malignant plasmas. Although this could not be demonstrated in the present population study substantial evidence has been obtained from other Danish studies in the 1914 population: smoking was related to symptoms (12), ten year incidence of CVD, peripheral arteriosclerotic disease (31), pulmonary function (1) and changes in pulmonary function. This underlines the importance for health of this very common habit. However, smoking is of low specificity has been used as an argument against the causality of smoking. It has then been proposed that impaired pulmonary function is a common denominator for the diversity multiplicity and relative non specificity of the effect of smoking. Morning cough, age 50 and FEV_{1.0} were significant risk factors for death of all causes in the 1914 population (1). Smoking was probably partly a risk factor for death through the effect on pulmonary symptoms and function. The coefficients for smoking were reduced though still significant when FEV₁ was introduced in the analysis of other risk factors of time to death. Pulmonary function was best in never smokers gradually decreased with increasing exposure. Smokers of more than one pack a day were a small group and selected by resistance to the harmful effect of smoking on the respiratory system and in taking up the habit. Ex smokers benefited from giving up smoking (8, 19) and more so as time passed. Ex smokers 1964-73 may have quit smoking because of symptoms due to it.

A strong relationship between cigarette consumption and FEV₁ (corrected for body build by dividing by height) persisted when allowance was made for possible confounding variables such as sex and

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Coronary Ischaemia and Occlusion in Giant Cell (Temporal) Arteritis

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ABSTRACT Reports of coronary artery involvement in giant cell (temporal) arteritis-polymyalgia rheumatica (GC(T)A-PMR) together with other arteries arising from the aorta have been numerous over the past 40 years but on this the literature has been virtually silent. This article summarises that evidence and records additional patients from a large group of cases with GC(T)A-PMR and ischaemic heart disease observed since a previous report in 1960. The disorder illustrates the benefit from corticosteroids and the hazards of non diagnosis and premature cessation of such treatment. It seems that patients with arteritic IHD (and claudication) are being identified before or after death. Possible reasons for this oversight by clinicians and logs are offered and suggestions are made regarding points in history taking and important signals which may help to alert the clinician. Is autopsy evidence from Malmö, Sweden that prevalence of GC(T)A-PMR is much higher than is suspected on clinical grounds.

and angina pectoris myocardial infarction giant cell temporal arteritis coronary arteritis
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It is well known that coronary arteries are as likely to be involved by giant cell (temporal) arteritis (GC(T)A) as the large arteries arising from the aorta is not rarely involved. Much of the evidence of involvement is derived from a previous communication (4¹) in which it was suggested inter alia that the cardiac arterial presentations of the disorder were likely to be more common than was recognised. It may be of clinical interest that this observation based on clinical history and supported by clinical measurements was unacceptable to an assessor at the time but on appeal the article was published. It was also suggested on clinical evidence (39-4²) GC(T)A and polymyalgia rheumatica (PMR) phases of the same disorder. This too pro-

voked controversy (7-43-49) but the hypothesis was later confirmed by positive biopsy of apparently normal temporal arteries in PMR (1) and of large arteries of the hip and shoulder girdles (70). All important studies since have been in accordance with these findings.

In 1960 Hughes and I wrote. Diagnostic failure in GC(T)A is due to many diverse presentations some of which are little known and to continued acceptance of a too rigid profile of the disease. The passage of 20 years has seen greatly improved recognition of the temporal and polymyalgia phases with the result that much pain and blindness have been prevented. However other manifestations of GC(T)A continue to go unsuspected and untreated in particular arteritic ischaemic heart disease (IHD), claudication and aortic aneurysm. The literature and personal experience also suggest that the gastrointestinal (7-16-41-4²) and psychiatric presentations (8-16-4²-50) are being overlooked as well.

Regarding arteritic IHD with which we are mainly concerned here the specialist cardiological literature with one exception (15) has remained silent whereas reports in the literature of general medicine and pathology have been numerous ever since Gilmore's report in 1941 (14) of the involvement of the aorta and its major branches by GC(T)A. It must therefore be assumed that cardiologists are either seeing the disorder but not diagnosing it or are diagnosing it but not reporting it.

PATIENTS FOR THE PRESENT STUDY

The writer's experience of patients with IHD and histologically proven GC(T)A and of other patients in whom

Abbreviations GC(T)A giant cell (temporal) arteritis
PMR polymyalgia rheumatica IHD ischaemic heart disease MI myocardial infarction

biopsy was not sought or obtained has been extensive since the previous report in 1960 when 9 out of 76 patients had IHD. Of over 100 cases seen over 20 years 9 have been selected to illustrate various aspects of presentation responses to treatment and hazards of relapse when corticosteroids are reduced prematurely either through over-optimism or inadequate supervision. Three cases with positive arterial biopsies are reported in detail six are described more briefly. In the latter six patients diagnosis was made on clinical grounds supported by laboratory findings in only two of these was arterial biopsy performed. It was positive.

THREE CASE HISTORIES

Case I

A married woman of 62 on 6.3.75 presented with angina of effort 2 months after 50 m and also claudication. She had had terrible buttock pain in Dec. 1978. Only after close questioning did she admit left jaw pain for 9 days. On examination left carotid artery tender and facial arteries thick and non-pulsatile. Bruit over left carotid and left brachial artery bruit to the elbow. ESR 126 mm Hb 10.3 g/100 ml. Increased α and γ globulin. Facial artery biopsy positive for arteritis vessel thrombosed but giant cells absent. ECG ischaemic S-T depression in V3-V4 and a Q3. Treatment with prednisone 10 mg t.i.d. was started on 6.3.75 with dramatic improvement the patient was able to do stairs and 250 m up hill after only 5 days of treatment. Discharged 21.3.75 on prednisone 5 mg t.i.d. ECG 21.3.79 had reverted to normal ESR 21 mm.

The patient has been observed for 4½ years until time of writing. On 4.8.75 a mild relapse of angina pectoris ESR at that time 35 mm. Prednisone increased to 12.5 mg. In Aug. 1976 she said she was so fit my husband cannot keep up with me. Left brachial bruit was still present but on 8.4.79 it could not be heard. She then had occasional angina of effort up hill in cold weather but no claudication. Resting ECG normal.

The patient initially denied mourning but then admitted shock and grief at the loss of two close friends six months (confirmed by husband) and much later casually mentioned that her mother had committed a particularly nasty suicide having escaped from the mental hospital where she had been admitted after finding the patient's sister dead in the lavatory. The patient had been unable to mourn either at the time.

Case II

A man of 69 referred with angina pectoris and claudication of 3 months duration on 9.10.78 by a doctor who had successfully treated temporal arteritis 15 months previously. Prednisone had been tailed off after 8 months. Examination facial arteries thickened bilateral brachial and iliac artery bruits right fundus ischaemic ESR 76 mm α_2 globulin + resting ECG normal. Temporal artery biopsy internal elastic lamina destroyed intimal proliferation lumen virtually occluded wholly compatible with arteritis 14 months previously. Treatment with prednisone 30 mg/day was started. In 3 days he reported that he had resumed work as a gardener to a large factory

without angina or claudication. Two months later still present ESR 8 mm/h. Prednisone gradually to 10 mg/day.

The patient did not attend follow-up on 1.12.78 of snow but on 15.5.79 he said that he had reduced the dose to 5 mg/day 3 weeks before and some time later to take it. ESR 94 mm ECG showed S-T depression and V5. On prednisone 5 mg t.i.d. his angina remitted and on 20.8.79 he was "very well". ESR arm bruits still present but iliac bruits absent.

Mourning first denied by the patient was revealed by his wife. His youngest daughter had been live with a married man six months before onset of temporal arteritis. He would have nothing to do with it but his wife could not bear to lose her joy. Part of this led to a rift between patient and wife and by loss of her as well as a replacement of the loss of a mother when he was a child. Briefly his wife expressed his mourning and brought the spouses together.

Case III

A married woman of 73. Angina of effort rheumatologist 2 years previously. "Tracer". When seen on 19.1.78 she was breathless and after 50 m. She had had jaw pain chewing for 1½ weeks had lost 12 kg. Left facial artery thick and some lateral brachial bruits to elbow and over femoral to the knee. ESR 22 mm increase in 10 days. Facial artery biopsy destruction of internal elastic lamina with fibroblastic intimal thickening and a minute lymphocytic foci in media and few giant cells. The artery finally disappeared in 10 days on prednisone and gained her lost weight.

Mourning was anticipated her husband was very ill with his prostate. She had feared he had died and that she would lose him as she had lost him much earlier. Her feelings for both were ambivalent.

BRIEF NOTES ON SIX CASES

Case IV

A single woman of 67 had temporal arteritis (biopsy in 1974). Corticosteroids were reduced and cessation of ESR insufficient. She relapsed with angina and claudication in 1977 with an ESR of 18 mm. Over eight carotid and left subclavian arteries. Increase in corticosteroids improved extremities from 50 to 150 m however her ESR remained high. Head pains recurred during reactivation of her mourning reaction ESR 93 mm. Unfortunately private action was not taken and the patient was dead three months later with the presumptive diagnosis myocardial infarction (MI) or more remotely as section.

Case V

A married woman of 69 referred in 1974 with angina gardening or walking 50 m. PMR responded to 10 mg in 1972 when ESR was 125 mm. She now had brachial bruits ESR was 53 mm. Left bundle branch

Autopsy histology positive for GC(T)A in coronary arteries

Coronary C = carotid T = temporal A = aorta R = radial Re = renal Sc = subclavian Br = brachial Bas = basilar
Op = ophthalmic Cl = ciliary Vert = vertebral S mes = superior mesenteric

Case	Year	Clinical presentation	Extent of arterial involvement demonstrated
1	1935	Temporal acute blindness	Co and Ca
2	1945	Polymyalgia head pain blindness confusion	T Co ARF S mes Re
3	1951	Head pains MI	A Co 1 aces Sc
4	1951	Cerebral thrombosis rheumatism	A Br Ca Bas Co ovarian
5	1954	Polymyalgia acute blindness	T A Ca Co
6	1955	Head pains blindness fever MI	T Op Co c rcle of W J s
7	1956	Head pains PUO acute blindness MI	T A Ca Co lingual Op
8	1959	Head pains fever acute blindness MI	T Ca Vert Op Cl
9	1960	Head pains acute blindness MI	T A 1 aces Ca Co Op Cl
10	1967	Polymyalgia head pains blindness MI	Op Ca Co
11	1968	Aortic aneurysm rupture	Lingual Ca A Co
12	1968	Polymyalgia acute blindness	T Co Ca A Sc S mes
13	1973	-	Co in 3 of 6 cases of GC(T)A PVR examined
14	1975	Polymyalgia abdominal pain aortic aneurysm cardiac arrest	A Co and all main aortic branches examined

pectoris responded to restoration of prednisone 15 mg/day. At last follow-up on 31.8.78 ESR 8 mm/h. Angina left brachial bruits still present. Mourning prior to onset and unresolved through course of

75-year-old woman of 75 admitted with proven MI in 1968. She was hampered by fever and pericarditis for 3 months. ESR 9 mm/h. She denied previous headache or head pains but later admitted "White" in my head. She had misheard her doctor's diagnosis of arteritis but had responded to prednisone when he emigrated. She stopped taking drugs as she did not want to trouble or face his reintroduction of prednisone led to immediate relapse. Mourning prominent at outset and in relapses.

75-year-old woman who never really got over the loss of her husband but had coped with the support of a cousin "more sister". A few months after the shock of losing the husband in 1977 she suffered severe head pains and tender joints for 17 months. In 1975 she was referred with severe head pains during which her aorta was tender, right sternal throb and barely pulsating. Blood was drawn and electrophoresis and her doctor was advised to initiate treatment with prednisone. He replied that patient had had a major coronary on the evening of 75 (the day after she was seen) and died later that afternoon. Prednisone could be started (ESR 71 mm/h and x-ray). Psychological mourning had been intensified just prior to onset of PVR.

75-year-old woman of 69 was referred in 1977 with angina. She had to stop every 50 m. The onset occurred 2

months after an alienation from a favourite daughter whose marriage and departure to a distant city she had been unable to accept. Her abdominal aorta was palpable and tender with a bruit. ECG was ischaemic and ESR 34 mm/h. Five days after a trial with prednisone she was able to walk further and her hands and feet felt warmer. Six months later she was able to do a mile without angina. At follow-up in 1979 she remained well on a small dose of prednisone and had an ESR of 18 mm/h.

Case IV

A widow of 76 who had declined in health since nursing her husband in his terminal illness the previous summer was admitted with left heart failure and confusion in April 1966. Temporal arteries were thick and tender. Biopsy positive ESR 54 mm/h. Through her confusion she told of severe head pains before her husband's death and thereafter exhaustion, muddled-headedness and angina. Treatment with prednisone was started 11 days after admission and brought about a dramatic improvement in her confusion and refractory cardiac failure as well as ECG and ESR. On postal follow-up her daughter said she had remained well for 12 months but had become confused again when prednisone had been reduced to 4 mg/day. When admitted later to another department her prednisone was promptly stopped despite an ESR of 83 mm/h and the patient died shortly after a series of strokes.

DISCUSSION

Table I summarises reports of histologically proven GC(T)A in the coronary arteries and other arteries also affected and chief clinical presentations.

The following references firstly relate to reports of patients with histologically proven GC(T)A

PMR who died of MI within 6 months of diagnosis and who were not autopsied (9/30/47) and secondly to reports of myocarditis or death from myocardial failure in patients with GC(T)A (16/29/37/38). Lastly there are reports of patients surviving with histologically proven GC(T)A-PMR with angina pectoris or associated MI (6/33/35/42/48).

At present the percentage of patients in whom GC(T)A-PMR coronary arteritis affects the quality or expectation of life is unknown. Personal experience suggests that the answer may be frequently especially in partly treated or inadequately controlled cases just as dissecting aneurysm of the aorta has been shown to be a complication to be reckoned with for the same reason by Klein et al. (31). They recorded aortic rupture in 3 out of 34 patients with clinical evidence of arteritis of the aorta and main branches.

Other unknowns are the real incidence of GC(T)A-PMR in the general population and in how many cases the disease continues to smoulder after initial treatment and how many of these die of MI or myocardial insufficiency. In a disorder with such a broad spectrum of presentations (42) some even now rarely recognised such as aortic regurgitation (25/42) and affecting especially the stoical and bereaved (40/42) it is not surprising that figures of incidence and prevalence derived from clinical data (17/21/27/28/36/44) fall far short of the most substantial autopsy evidence to date. Östberg (37) reporting from Malmö found GC(T)A in 1.4% of 560 consecutive adult autopsies in which she examined a transverse section of the aorta 5 cm above the aortic valve. In a subsequent series of 889 autopsies in which an additional section of aorta from the level of the diaphragm and also sections of the temporal artery were examined the finding of giant cell arteritis rose to 1.7%. Östberg concluded that this is a minimum figure and since the lesions are patchy there is a considerable chance that a few small specimens will miss the arteritic changes. Her comprehensive studies confirm the importance of intimal changes presenting as pearly cushion-like areas and in the later stages progressing to fibrosis of the intima and adventitia with shrinkage of the lumen. The classical changes in GC(T)A in the media require no emphasis but Östberg reiterates the warning that giant cells are "not a consistent finding" except during the phase of elastin degradation and adds: "The name of giant cell arteritis therefore seems but little appropriate but is probably too well established to be changed." As to how often arteritis in these patients presenting with IHD the evidence is scanty. Östberg found the coronary arteries involved in 3 out of 6 cases known to have GC(T)A-PMR previously but clearly further work still remains to be done. This present study requires a study comparable to Östberg's consecutive series of autopsies after MI and may be difficult because of the patchy nature of arteritis and the frequency of associated changes (37/42) some of which perhaps develop in response to the arteritic insult. Such a case relapse associated with mourning or mourning was observed closely over 1 year towards the end her aorta was almost obliterated.

Allowing for such difficulties certain others will ask why in view of Östberg's findings few more cases have not been removed at autopsy. One can only make suggestions. It may be significant that most of the autopsies showing coronary involvement were performed most of those patients were blind following the temporal arteritis and most had not received corticosteroids or corticotrophin which would probably have prevented blindness or if they did received probably for insufficient time to suppress the arteritis. Allowing 2-3 years lag between arteritic complication this fits closely to the generalisation of these remedies which in the UK for oral corticosteroids was about 1957. Personal evidence and the literature since 1960 show that the arteritic phase is now usually recognised and treated which is why arteritic blindness has become less common. It seems that what has happened is that what were formerly pathologists' patients tending to see are smouldering partly and poorly monitored cases in which the aorta and main branches including the coronary arteries have become gradually involved (41) the classic example despite close supervision a patient reduced his prednisone dose from 10 mg to 5 mg to relapse temporarily with an ESR of 60 mm/h but supervision been less close the outcome would have been MI.

So unless the histopathologist is alerted by clinicians when a patient with MI who has had or is thought to have had GC(T)A-PMR he is unlikely to look for it.

presence of obvious atheroma and thrombi to this hospital clinicians often do not know a patient admitted with MI has been treated with GC(T)A because the general practitioner does not think to mention it being unaware that early disease claudication and aortic dissection potential sequelae

Diagnostic pointers

Among the better histological evidence there are features which should alert the clinician that a patient presenting with MI or angina pectoris may have underlying GC(T)A (a) A history of head pain in the preceding year or two facial neuralgia or shoulder polymyalgia or claudication needs emphasis. However it is less well known that grief is likely to be in pathological mourning a key figure or surrogate and the surrogate may be a spouse child friend or pet. This observation made 20 years ago recurs in case after case as the rule rather than an exception (40-42) and is validated by independent observers of unedited videotaped interviews of 12 patients with GC(T)A. (b) The mourning reaction is usually protracted because of ambivalent feelings for the key figure(s). Grief is usually concealed until the patients are asked whether any of those they have lost are much in their thoughts. If (d) is 'X is never out of my thoughts' accompanied by a quivering lip or break in voice a point is added to the index of suspicion. (c) Malaise persisting beyond expectation and delaying recovery together with a raised ESR persisting for 3 weeks after MI and for which no other cause can be found often accompanied by raised albumin. (d) In the event of any of (a) (b) (c) a positive a most careful examination should be made for thickening and/or tenderness of temporal facial occipital carotid arteries and for much longer lengths of vessel than with normal notably to the elbow over the brachial arch and often from the iliacs to the knee over the femoral canal (18). The abdominal aorta itself may be tender (41) and aortic valve incompetence may be developed (25-42). If (d) is positive the clinician may decide to ask for a biopsy of facial or temporal artery bearing in mind that it may still be positive even in the presence of active GC(T)A involving the aorta coronaries and other branches. Patients may be reluctant to submit such sick people to such a relatively mild procedure as facial/

temporal artery biopsy especially if other evidence has been enough to decide on a trial of corticosteroids in any case.

The arteriographic studies of Klein et al (31) and Hamrin (17) clearly show the reason for the extensive bruits in GC(T)A-PMR. Whether improved coronary arteriography will help to differentiate arteritic areas from atheroma remains to be seen.

Diagnostic pitfalls

Not only may temporal and facial artery biopsies be negative in GC(T)A-PM (26-37-42) especially if giant cells are regarded as essential to diagnosis but in perhaps 3-5% of cases the ESR may also be repeatedly normal (6-16-35-42).

Since Bottiger and Svedberg's report (5) it is unfortunate that some generalists have been teaching that a raised ESR is a normal finding in the elderly. This doctrine is dangerous and it would be wise to regard it as non-proven until more clinical evidence is available than provided in the above study. My experience supported by Eckerstrom (12) is that elderly people have a normal ESR unless there is cause for it to be raised such as infection neoplasm myeloma silent large peptic ulcers subacute bacterial endocarditis rheumatoid arthritis SLE Paget's disease and last but not least GC(T)A.

Mourning and immunological response

GC(T)A-PMR is now thought by many to be an immunological disorder and Bartrop et al (4) showed that thymic mediated immunity was reduced during mourning. This may explain why grief and mourning sometimes even in anticipation of the loss of a key figure are so relevant in this disorder (42-47). Because these patients have difficulty in showing emotion some tend to endure their symptoms along with their grief until they are very ill and even dead (cases VII and IX are examples).

Indeed the physical disability coming on top of grief not infrequently leads to attempted suicide. Cases of GC(T)A-PMR coming to diagnosis may only represent the tip of an iceberg of undetected disease in the elderly.

CONCLUSION

It seems likely that many patients with arteritic IHD are not receiving the help they should and the same applies to arteritic claudicants. The object of this

article is to pose the question whether it is better to accept the evidence we have which is substantial but imperfect and try to identify more cases of GC(T)A affecting the aorta and great vessels including the coronary arteries and treat them during life or to adopt a more scientific stance and wait for the results of more definitive autopsy studies

Cases I-III are examples of what can be done for severe angina pectoris and/or claudication when due to GC(T)A. Temporal/facial artery biopsy was positive in each but head pain and polymyalgia were minimal in cases I and II at the time of the onset of angina pectoris and claudication but had occurred 15 months previously in case II

Cases IV-VI-VII-IX are examples of the cardiac price likely to be paid for not diagnosing and treating PMR-GC(T)A soon enough or to monitor and control reactivity in a diagnosed case

The following diagnostic traps need to be stressed (a) Normal biopsy does not exclude the possibility of GC(T)A (b) Intimal proliferation without giant cells may predominate over the more commonly recognised changes (c) Arteritic lesions are patchy (d) ESR may be normal in a few cases (e) Severe anaemia can also cause the characteristic arterial bruits

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Zinc Balance before and during Treatment with Bendroflumethiazide

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ABSTRACT The present study gives full balance of zinc in two healthy individuals before and during treatment with chlorthalidone. Positive zinc balances were noted in both subjects without medication. During chlorthalidone treatment the balance changed in a negative direction. Thus the balance for slightly increased serum zinc values which were observed during diuretic treatment in spite of increased urinary losses of zinc cannot be explained by increased zinc absorption. Depletion of zinc in tissues seems likely.

Key words: zinc balance, chlorthalidone.
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Urinary excretion of zinc has been found to be increased during therapy with hydrochlorothiazide (2) and furosemide (6, 7). Furosemide and bumetanide have been found to raise the urinary zinc concentration. However, with regard to the large urine volumes produced during such medication, the amount of zinc excreted is augmented (5, 10). On the other hand, serum zinc values in patients on diuretics are not even slightly raised (2, 9). There are at least two possibilities to explain this circumstance: intestinal absorption of zinc might be raised during thiazide therapy or tissues may be depleted.

The aim of this study was to elucidate this question by investigating the zinc balance in two healthy individuals before and during thiazide therapy and also during thiazide therapy with zinc supplementation.

SUBJECTS AND METHODS

Two healthy female technical assistants, 29 and 32 years of age, were subjected to the study. They were kept on a constant diet prepared in the metabolic ward and their urine and faeces were collected for 8 metabolic balance periods of 5 days. The conventional carmine labelling technique was applied to separate the periods (3). The loss

of zinc in urine and faeces during each balance period was determined on aliquots of urine and homogenates of faeces with the aid of atomic absorption (Varian Techtron Model 1100). The intake of zinc was determined by analysis of homogenates of duplicates of the daily menu. The diet of subject A was calculated to contain 101 g protein, 94 g fat and 248 g carbohydrate. Corresponding values for the diet of subject B were 81, 93 and 235 g.

The zinc balance studies in subjects A and B were performed in the following way. Periods I and II: no medication. Periods III and IV: 5 mg bendroflumethiazide (Salures[®]) and 1.5 g potassium chloride daily. Period V: same as periods III and IV plus 7.5 mg Zn⁺⁺ as a chloride solution twice a day. Period VI: 10 mg bendroflumethiazide, 1.5 g potassium chloride and 7.5 mg Zn⁺⁺ as a chloride solution twice a day. Periods VII and VIII: Same as period VI but the Zn supplementation was replaced by 50 mg Mg⁺⁺ as a chloride solution.

RESULTS

Table I presents the balance data of both subjects. Without any medication (periods I and II) the zinc balances were positive in both. The Zn intake was 14 mg in subject A and 12.5 mg in subject B. 69% appeared in faeces in subject A and 70% in subject B during periods I and II. A correction of the balances was made with regard to calculated losses of Zn by skin. The loss of zinc by skin (sweat and desquamation) is estimated to be 2.8 mg daily according to recently published data (4).

During thiazide therapy without Zn supplementation (periods III and IV) the urinary zinc excretion increased by 29% in subject A and by 55% in subject B compared to their urinary excretion during periods I and II. The faecal excretion during periods III and IV was 82% in subject A and 73% in subject B. The corrected mean Zn balances during periods III and IV were negative in subject A and about zero in subject B. During thiazide therapy with Zn supplementation the Zn balances were strongly positive in both subjects. The urinary excretion of Zn during Zn supplementation rose

Table 1 Zinc balance (mg/24 h)

Period	Treatment	Daily intake	Excretion		Balance	Comment
			Faecal	Urinary		
<i>Subject A</i>						
I	None	14.0	10.4	0.25	+3.35	
II		14.0	8.4	0.22	+5.38	
Mean for I and II		14.0	9.4	0.24	+4.36	
III	5 mg bendroflumethiazide	14.2	12.4	0.32	+1.48	
IV	+ 1.5 g KCL	14.2	10.9	0.29	+3.01	
Mean for III and IV		14.2	11.7	0.31	+2.19	
V	5 mg bendroflumethiazide	27.4	23.6	0.28	+3.52	
VI	+ 1.5 g KCL + 15 mg Zn					
	10 mg bendroflumethiazide	30.1	20.5	0.30	+9.3	
	+ 1.5 g KCL + 15 mg Zn ⁺⁺					
Mean for V and VI		28.8	22.0	0.29	+6.51	
VII	10 mg bendroflumethiazide	14.8	15.1	0.36	-0.66	
VIII	+ 1.5 g KCL + 50 mg Mg ⁺⁺	14.9	11.7	0.41	+2.79	
Mean for VII and VIII		14.9	13.4	0.39	+1.11	
<i>Subject B</i>						
I	None	12.5	8.3	0.41	+3.79	
II		12.5	9.0	0.46	+3.04	
Mean for I and II		12.5	8.6	0.44	+3.46	
III		12.6	8.7	0.63	+3.27	
IV		12.6	9.7	0.73	+2.17	
Mean for III and IV		12.6	9.2	0.68	+2.72	
V		27.1	19.3	0.71	+7.09	
VI		27.1	20.7	0.76	+5.64	
Mean for V and VI		27.1	20.0	0.74	+6.36	
VII		12.4	13.1	0.63	-1.35	
VIII		13.8	14.5	0.60	-1.30	
Mean for VII and VIII		13.1	13.8	0.63	-1.33	

only slightly in subject B and not at all in subject A. The percentage excreted by faeces was about the same as during periods III and IV. When Zn supplementation was replaced by Mg supplementation the Zn balances were strongly negative in both subjects and the urinary Zn excretion remained at about the same level. The mean amount excreted in faeces during periods VII and VIII exceeded the intake in subject B and amounted to 90% in subject A.

DISCUSSION

It has been suggested that therapy with thiazides and other diuretics may cause deficiency not only of potassium and magnesium but also of zinc (1-9).

Raised urinary losses of potassium and magnesium during medication with thiazides and furosemide has been well known for a long time. Potassium supplementation is usually given. Urinary losses of zinc have been found during therapy with chlorthalidone (6, 7, 10), H₂O, chlorothiazide (2, 10), bendroflumethiazide, furosemide (5, 10), bumetanide and frusemide (10). The increased losses persisted after 6 months' treatment with hydrochlorothiazide (10). The serum zinc levels, however, did not fall during therapy (2, 8, 9). On the contrary, in a few patients treated with different diuretics for less than six months the serum zinc level was higher than in 96 apparently healthy subjects.

as explained by the effect of diuretics on volume. The explanation of normal or high Zn levels in spite of increased losses in urine and diuretic therapy may be either that zinc is lost from tissues causing an intracellular zinc deficiency or that zinc absorption is augmented in vivo. The present study clearly shows that absorption was not increased during medical bendroflumethiazide in two healthy subjects. In the contrary, the zinc balances were in a negative direction in both subjects on thiazides. The urinary Zn excretion in both thiazides were administered as reference (7-10). However, the increase in output is far too small to completely explain the negative balances. The percentage of zinc intake appearing in faeces was increased from 70% in subject A and from 70 to 74% in subject B. The decrease in Zn absorption might be due to the interaction with bendroflumethiazide or to the interaction with potassium chloride. The zinc balances were strongly positive when Zn intake was given in periods V and VI. In subject A the percentage of Zn intake appearing in faeces was not higher during period VI when 10 mg bendroflumethiazide was given than during period V when only 5 mg was administered. In subject B the faeces contained slightly more Zn during period VI than during period V. When magnesium supplementation was given during periods VII the zinc balances were strongly negative in both subjects. More Zn than ingested appeared in faeces in subject B. The explanation may be part of the extra Zn load absorbed during periods V and VI was now excreted to the bowel. In conclusion the Zn balances in the two healthy subjects changed in a negative direction during treatment with bendroflumethiazide combined with potassium supplementation. When both potassium and magnesium supplementation was given the

balances were strongly negative in both subjects. It might be that additional intake of both potassium and magnesium decrease the intestinal absorption of zinc. The increased urinary losses of Zn during treatment with diuretics with maintained or increased serum Zn levels cannot be explained by increased zinc absorption. Thus it seems probable that tissues are depleted of zinc during treatment with diuretics.

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Tissue Zinc at Autopsy— Relation to Medication with Diuretics

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ACT Diuretic treatment has been shown to substantial increase in the urinary output of water, the serum zinc levels remain normal raised. In the present study tissue zinc was in liver, kidney and skeletal muscle tissue in consecutive autopsies. In 90 of the cases nuclei and aortic tissue were also studied. The values were correlated to previous treatment with diuretics. A lower liver zinc level was observed in a group of patients who had been on diuretic treatment for more than 6 months compared to the group without treatment, the difference being highly significant. The skeletal muscle zinc was also low in patients who had been on diuretic treatment. With regard to the metabolic functions of zinc, the depletion of this element after diuretic treatment may be of clinical importance.

di zinc diuretics liver kidney skeletal muscle
muscle aorta
Acta Med Scand 208 269 1980

Urinary zinc excretion has been found during treatment with hydrochlorothiazide (5-14) bendroflumethiazide (14) chlorthalidone (10-11-14) furosemide (7-14) bumetanide (14) and frusemide (12). However the serum level of zinc in patients treated with diuretics for more than 6 months was found to be normal or raised (13). A study of zinc during diuretic therapy demonstrated that increased zinc absorption was not a sufficient explanation of maintained or increased serum concentration of zinc despite increased urinary losses. A better explanation may be that zinc is mobilized from tissues causing an intracellular zinc deficiency.

The aim of the present study was to investigate zinc content in skeletal muscle, heart muscle, kidney and aorta in autopsies in patients who had and who had not been on diuretics.

MATERIAL AND METHODS

Skeletal muscle, liver and kidney tissue were collected from 147 consecutive autopsies. From the last 90 of these autopsies a piece of aorta and myocardium were collected as well. The skeletal muscle tissue was taken from the muscle of the right lobe, the kidney tissue from the middle of the lateral part of the right kidney, the heart muscle tissue from the anterior wall of the left ventricle and the aortic tissue from the ascending part of the aorta. The samples were kept at -20°C until required for analysis. Zinc was determined by atomic absorption (Varian Techtron Model 1100). The tissue samples were dissolved in 2 ml fuming H_2SO_4 and 5 ml H_2O_2 .

The 147 autopsies were divided into three groups based on causes of death. Group A consisted of 49 patients with various causes of death, group B of 62 patients whose death was caused by coronary artery disease and group C of 36 patients with various tumors as the cause of death.

All records were studied retrospectively with regard to medication with diuretics. If information about medication was missing or incomplete in the records, the relatives and/or the house doctor were contacted. Based on the diuretics administered (thiazides and/or furosemide) the material was divided into three groups. Group I: No medication with diuretics could be traced. This group consisted of 46 autopsy cases, 11 of whom had died from coronary artery disease, 16 from various tumors and 19 from other causes. Group II: Diuretics had been administered for less than 1 month. In this group there were 36 cases, 30 of whom had died from coronary heart disease, 13 from various tumors and 9 from other causes. Group III: Diuretics had been given for more than 6 months. This group consisted of 45 cases, 20 of whom had died from coronary artery disease, 3 from tumors and 22 from other causes.

In patients who had had diseases known to affect the zinc content in various organs, the zinc values from this particular organ were excluded from the study. Thus 26 liver Zn values, 6 in group I, 14 in group II, 6 in group III were excluded due to liver cirrhosis and/or tumor metastases of the liver. Zinc in kidney was excluded in 35 autopsied patients with regard to kidney diseases, most often due to acute cystitis.

Student's *t* test was applied in the statistical calculations.

Table 1 Mean values of zinc ($\mu\text{g/g}$ wet weight) in different organs from autopsied patients with earlier medication with diuretics

Group A = various causes of death group B = death from coronary heart disease group C = death from

Group	Without diuretics	Diuretics <1 month	Diuretics >6 months
Liver tissue			
A	59.9 \pm 33.6 (n=16)	92.0 \pm 63.2 (n=3)	51.3 \pm 33.7 (n=19)
B	75.3 \pm 28.1 (n=11)	67.4 \pm 24.6 (n=26)	48.3 \pm 17.5 (n=18)
C	108 \pm 52.7 (n=12)	63.5 \pm 27.8 (n=11)	41.6 \pm 47.8 (n=2)
A+B+C	79.2 \pm 45.1	68.7 \pm 27.9	49.6 \pm 30.1
Kidney tissue			
A	35.3 \pm 6.5 (n=12)	39.4 \pm 16.9 (n=7)	38.2 \pm 16.7 (n=14)
B	31.6 \pm 9.5 (n=7)	35.6 \pm 13.3 (n=30)	30.4 \pm 7.7 (n=14)
C	49.5 \pm 24.0 (n=12)	37.4 \pm 9.8 (n=12)	24.0 \pm 7.2 (n=9)
A+B+C	39.6 \pm 19.0	36.8 \pm 12.8	33.0 \pm 14.3
Skeletal muscle tissue			
A	49.5 \pm 12.3 (n=18)	56.5 \pm 17.5 (n=9)	48.2 \pm 11.5 (n=22)
B	58.5 \pm 14.4 (n=11)	52.8 \pm 13.8 (n=30)	48.7 \pm 9.9 (n=20)
C	54.8 \pm 16.0 (n=16)	55.5 \pm 10.4 (n=17)	50.0 \pm 33.3 (n=2)
A+B+C	53.6 \pm 14.4	54.2 \pm 13.4	48.0 \pm 10.5
Heart muscle tissue			
A	27.2 \pm 4.3 (n=12)	30.2 \pm 4.7 (n=4)	28.7 \pm 6.3 (n=11)
B	29.8 \pm 6.2 (n=8)	27.7 \pm 7.1 (n=20)	26.7 \pm 7.3 (n=17)
C	34.2 \pm 13.3 (n=9)	28.7 \pm 5.3 (n=8)	17.1 \pm 25.7 (n=9)
A+B+C	30.1 \pm 8.7	28.1 \pm 6.3	27.2 \pm 6.8
Aortic tissue			
A	25.5 \pm 7.8 (n=13)	32.4 \pm 13.8 (n=4)	28.2 \pm 18.8 (n=11)
B	28.5 \pm 14.5 (n=10)	24.5 \pm 10.1 (n=20)	21.0 \pm 4.5 (n=17)
C	25.6 \pm 4.8 (n=9)	29.9 \pm 19.1 (n=8)	28.3 \pm 22.8 (n=2)
A+B+C	26.5 \pm 9.6	26.8 \pm 13.2	24.5 \pm 13.0

RESULTS

The zinc values in the liver kidney skeletal muscle heart muscle and aortic tissues are presented in Table 1

The mean zinc value in liver tissue from autopsied patients who had not taken any diuretics was 79.2 $\mu\text{g/g}$ and from those who had been taking diuretics for more than 6 months 49.6 $\mu\text{g/g}$. The difference between these two values is highly significant. If only group B (patients who had died from coronary artery disease) is selected among patients who had not taken any diuretics and compared to group B among patients who had taken diuretics for more than 6 months the difference in liver zinc is still significant ($p < 0.0025$). There was no significant difference between the group without diuretics and the group who had taken diuretics for less than 1 month. Within the group of patients who had not taken any diuretics group C (patients who had died from tumors) had more zinc in the liver

than group A (patients who had died from various causes). Within the groups of patients who had taken diuretics for less than 1 month and for more than 6 months there were no significant differences between groups A, B and C.

No significant differences in kidney tissue were observed with regard to previous diuretic medication (Table 1).

In skeletal muscle the zinc content was significantly lower ($p < 0.025$) in patients who had taken diuretics for more than 6 months than in patients who had not been taking diuretics. If group I (without diuretics) plus group II (diuretics for less than 1 month) is compared with group III (diuretics for more than 6 months) the degree of significance increases ($p < 0.005$).

No significant differences in zinc content were observed in heart muscle and aortic tissue were observed with regard to the different diuretic groups. There was, however, a tendency towards lower values in group C (patients who had died from tumors) in the diuretic group.

DISCUSSION

Most prominent finding in the present work is a highly significant reduction in liver zinc in patients who had been taking diuretics for more than six months. The mean value and standard deviation for liver zinc in patients who had not taken diuretics are in excellent agreement with values in 3 patients who had died from various diseases whose livers were histologically normal (2). Liver zinc is known to be strongly reduced in cirrhosis and in tumors and tumor metastases (2, 8). This was also true in the present study and 26 liver Zn values were excluded from the study. High liver zinc values were noted in 10% of group I viz. patients who had died from cancer in other parts of the body than the liver. This is also in agreement with other studies. The mean liver zinc value in group III (diuretics only) was very low and in agreement with data on liver cirrhosis (8). Previous studies have revealed that treatment with diuretics causes much more extensive losses of zinc than treatment with loop-diuretics or thiazides. In the present study it was however not possible to correlate the reduction of tissue zinc with the use of different diuretics because too many patients had used more than one type of diuretics. It has recently been pointed out that the serum zinc concentration is not always a good indicator of zinc deficiency (1). There is evidence that the liver zinc concentration may be a more sensitive factor. In patients receiving zinc-deficient diet the liver zinc content in whole blood was reduced after 4 weeks serum zinc however remained at normal levels (3). In patients with total starvation in man the zinc balances were strongly negative but the serum zinc levels remained normal (6, 9). The liver zinc was however not studied in these patients. Further after ligation of the pancreatic duct in rats the liver zinc was strongly decreased but the serum zinc remained normal (1). It seems as if the serum zinc does not decrease until most of the zinc stores primarily in the liver are reduced. In the present study the zinc concentration in skeletal muscle was also significantly reduced. This indicates that the reduction of tissue zinc in patients on long term diuretics is more extensive than only restricted to the liver. If the zinc re-

duction in tissues during diuretic treatment causes impairment in the function of various organs or not is unclear. Further studies are needed to answer this question. However taking into consideration that zinc is an essential element in human metabolism it is probable that the zinc losses are of clinical importance.

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Effects of Mefruside Treatment in Hypertension

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ABSTRACT Fifty-two middle-aged patients with essential hypertension were treated for five months with mefruside as the only antihypertensive. Blood pressure, heart rate, fasting blood glucose, cholesterol, triglycerides, uric acid, electrolytes were controlled regularly before and during treatment. Blood pressure normalized in 43 cases. The decrease was more marked in the first 3 months and their maximal response appeared later. Significant changes were seen in cholesterol and triglycerides. Serum uric acid levels increased significantly in both sexes ($p < 0.001$) but no patient developed gout. A significant decrease ($p < 0.001$) in serum potassium was seen only in one male, with no effect on diabetes mellitus. No side effects were observed. Mefruside is a valuable alternative to thiazide treatment, especially when hypokalaemia, glucose intolerance or gout have developed.

Key words: essential hypertension, non-alcoholic mefruside, metabolic effects, sex differences.
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Treatment of hypertension has attracted great interest, especially since the demonstration that normotension might be achieved in a large number of patients with severe hypertension (22). All primary screening trials are in progress in Sweden and the results indicate decreases in mortality as well as mortality (3). In the 40s and 60s diuretics of the thiazide type were used as the drug of choice. Treatment with β -blockers was introduced during the 70s and are now the drugs of choice in the Scandinavian countries together with diuretics. The early use of β -blockers saw the introduction of low-dosage thiazide treatment (3) but its long-term evaluation is not yet clear. Side effects from saluretics with glucose intolerance and possible development of diabetes, gout and hypokalaemia are well known and lead to withdrawal of the drug in 5-15% of patients (8, 10, 14, 17, 19, 21, 26). New data may indicate that long-term side effects can appear on

treatment with thiazide diuretics. In two controlled studies (1, 2) cholesterol and triglycerides were significantly elevated during treatment with chlorothalidone and hydrochlorothiazide but these results are contradicted by others (16). Spironolactone is a valuable alternative to thiazide treatment, especially when hypokalaemia, glucose intolerance or gout have developed.

Mefruside (Baycaron®) has been available on the Swedish pharmaceutical market since 1971 (7, 8, 19). Mefruside is chemically closely related to thiazides but it has been claimed that intracellular potassium and magnesium depletion is less pronounced (8). It remains to be confirmed whether these intracellular findings prevail during long-term treatment. Several authors claim that treatment with mefruside gives rise to less side effects as regards glucose intolerance, hypokalaemia and hypomagnesaemia (6, 9, 19, 26). The antihypertensive effect of mefruside in a single dosage of 25 mg daily has been well documented (7, 8, 21).

In view of the reported significant increases in cholesterol and triglycerides during therapy with thiazides and thiazide-like diuretics, this investigation was undertaken to study partly the metabolic effects of mefruside and partly the sex-related antihypertensive effect.

PATIENTS

Only 40-65 year-old patients with a diastolic blood pressure (BP) on three visits of ≥ 100 mmHg after 10 min in the supine position and a mean heart rate of < 90 beats/min were included in the study. The 52 patients (26 males, 26 females) were selected from five out-patient clinics in the city of Malmö (240 000 inhabitants). All had denied a history of alcohol overconsumption.

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Table 1 Blood pressures (mmHg) and heart rates (HR) (beats/min) before and during treatment with mefruside 25 mg once daily (mean \pm 1 S D)

	Males (n=26)						Females (n=24)	
	Supine			Erect			Supine	
	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP
Before therapy*	160.4 \pm 15.0	103.5 \pm 5.6	72 \pm 9	156.0 \pm 19.1	106.3 \pm 8.0	79 \pm 9	176.5 \pm 18.6	114.9
During therapy								
1 month	144.6 \pm 16.1	94.2 \pm 6.7	76 \pm 9	140.4 \pm 18.7	98.5 \pm 8.8	82 \pm 9	155.1 \pm 15.9	94.6
3 months	143.8 \pm 16.8	93.5 \pm 7.2	72 \pm 9	140.0 \pm 18.5	98.8 \pm 7.8	81 \pm 9	147.2 \pm 15.5	94.4
5 months	141.3 \pm 16.7	91.0 \pm 7.1	71 \pm 9	139.0 \pm 20.1	97.0 \pm 9.0	77 \pm 9	145.5 \pm 15.3	89.1
p (t test)	***	***	N S	***	**	N S	***	*

* BP before treatment was calculated as the mean of three visits

N S = not significant ** $p < 0.01$ *** $p < 0.001$

METHODS

The BP was measured with a calibrated mercury manometer by nurses. Diastolic BP defined as phase V according to Korotkoff was measured initially in both arms and at later controls in the arm with the highest diastolic pressure. The measurement was done after 10 min in the supine and after 1-2 min in the standing position together with heart rate. The treatment consisted of 25 mg mefruside (Baycaron®) daily during the breakfast. Controls after 1, 3 and 5 months of therapy were done during fasting conditions in the morning together with the following parameters: BP, plasma concentrations of sodium, potassium, creatinine, calcium, albumin, magnesium, blood glucose,

cholesterol, triglycerides and uric acid. They were controlled for glucose, protein and blood

If fasting blood glucose rose during treatment above the upper normal limit (5.6 mmol/l) serum sodium decreased to values below 3.5 mmol/l or potassium to values below 0.70 mmol/l , the determinations were repeated together with an oral glucose tolerance test ($1 \text{ g glucose/kg b.wt.}$). If this showed a positive result, mefruside administration was discontinued and a new tolerance test was carried out after two weeks. Potassium was given orally only if an established hypokalaemia was present.

The metabolic parameters were measured with the methods of the Central Laboratory at Blädd Hospital. The following normal ranges have been used: S-Na $131-151 \text{ mmol/l}$, S-K $3.5-5.0 \text{ mmol/l}$, S-Ca $2.20-2.60 \text{ mmol/l}$, S-Mg $0.70-1.10 \text{ mmol/l}$, S-crea $60-115 \text{ } \mu\text{mol/l}$, S-triglycerides $40-60 \text{ years } 0-4 \text{ mmol/l}$, S-cholesterol $< 7.70 \text{ mmol/l}$, fasting blood glucose $< 5.6 \text{ mmol/l}$, S-albumin $40-52 \text{ g/l}$.

RESULTS

Effects on blood pressure

Results for BP and heart rate are shown in Table 1. Systolic BP supine and erect before treatment was significantly higher in females ($p < 0.01$) but there was no significant difference in either the systolic or the diastolic BP in both supine and erect after 5 months of treatment. The decreases in both systolic and diastolic BP supine and erect during the first month and the two following months were significantly more pronounced in females than in males ($p < 0.05$ after 3 months $p < 0.01$). Heart rate supine position was similar in both sexes before control; it was 6-9 beats higher in the erect position ($p < 0.05$).

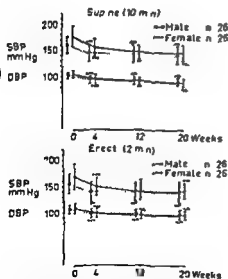


Fig. 1 Systolic (SBP) and diastolic (DBP) BP before and during therapy with mefruside 25 mg once daily. Note that each mean BP is compared with the previous control during therapy. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. = not significant.

mm Hg	
DBP	HR
109.8±9.8	87±10
99.1±8.5	83±11
94.6±6.1	80±10
95.5±7.7	87±10
N S	

standing systolic BP was lower on average supine in both sexes both before and during but the opposite applied for the diastolic BP.

Five months of treatment BP in both supine and standing position had decreased in all but two (3.5%). Seven patients (13%) still had a diastolic BP of >100 mmHg; they received additional therapy with β blockers after the study was completed. Two other patients had a diastolic BP of >100 mmHg but both had a supine diastolic BP and they continued with the same after the study. Both supine and standing reduced in 43 patients (82.7%) during mefruside treatment in a dose of 25 mg daily.

Metabolic effects

Results for the metabolic parameters are shown in Table II.

Blood glucose

One patient had an overt diabetes mellitus. As shown in Table II, no significant increase in fasting blood glucose occurred in males or females. In six males (3/4, 9/2) the fasting blood glucose rose to 5.6 mmol/l and they were examined with an oral glucose tolerance test (1 g glucose/kg). This was normal in all but one patient, a 50-year-old with a hereditary background for diabetes. His treatment with mefruside was withdrawn after five months; three weeks later a new glucose tolerance test was performed. The fasting blood glucose values in the five patients after 5 months of therapy

were normal. During the study the weight of the individual patient changed considerably and no significant correlation was found between weight and fasting blood glucose at any control. Neither was there any significant correlation between changes in serum potassium and blood glucose (12).

Serum potassium

Serum potassium decreased significantly in both sexes ($p < 0.001$). Two patients showed hypokalaemia; one of them with concurrent hyperglycaemia. One male had hypokalaemia (3.4 mmol/l) at the start of the study and it decreased to 2.9 mmol/l but he had a normal oral glucose tolerance test. After the study he continued with mefruside together with potassium tablets. He may have an endocrine type of hypertension but further investigation is not complete.

Serum uric acid

Serum uric acid increased in both sexes ($p < 0.001$). No patient developed clinical signs of gout during therapy. One patient had an increased value initially (530 μ mol/l) with a further increase during therapy to 601 μ mol/l and he was subsequently treated with allopurinol (Zyloric®).

Serum cholesterol

Serum cholesterol was measured before and after 5 months of therapy. No patient developed hypercholesterolaemia and the blood levels were unchanged in both sexes. Ultracentrifugation studies were not carried out.

Serum triglycerides

The serum triglycerides did not show any significant change. Five patients had an initial hypertriglyceridaemia; two returned to normal values and three were unchanged (25). There was a positive but non significant correlation ($r = 0.23$) between changes in weight and serum triglycerides.

Serum calcium

Serum calcium did not change significantly and no patient developed hypercalcaemia, which is known to occur during therapy with thiazides (11, 23, 24).

Plasma albumin

In males there was a significant drop in plasma albumin ($p < 0.05$) but not in females.

Table II Results from metabolic investigations of blood and changes in body weight during treatment with 25 mg mefruside daily (mean \pm 1 S D)

	Fasting blood sugar (mmol/l)	Potas- sium ⁺ (mmol/l)	Uric acid (μ mol/l)	Chol- esterol (mmol/l)	Triglyc- erides (mmol/l)	Calcu- m (mmol/l)	Age (y)
Females							
Before therapy	4.8 \pm 0.7	4.2 \pm 0.4	250 \pm 58	6.1 \pm 1.1	1.29 \pm 0.53	2.36 \pm 0.17	41
During therapy							
1 month	5.1 \pm 0.9	3.8 \pm 0.4	308 \pm 86	—	—	2.39 \pm 0.13	5
3 months	4.8 \pm 0.8	3.8 \pm 0.4	312 \pm 75	—	—	2.40 \pm 0.13	4
5 months	4.8 \pm 0.7	3.9 \pm 0.4	309 \pm 64	6.3 \pm 0.8	1.46 \pm 0.61	2.40 \pm 0.11	4
p (t test)	N S	***	***	N S	N S	N S	N
Males							
Before therapy	4.5 \pm 1.1	4.3 \pm 0.4	325 \pm 75	5.7 \pm 1.3	1.35 \pm 0.50	2.45 \pm 0.11	41
During therapy							
1 month	4.9 \pm 1.2	3.9 \pm 0.3	383 \pm 68	—	—	2.41 \pm 0.17	17
3 months	4.9 \pm 1.0	3.9 \pm 0.4	399 \pm 102	—	—	2.45 \pm 0.13	17
5 months	4.9 \pm 1.0	3.9 \pm 0.4	377 \pm 86	5.7 \pm 1.1	1.47 \pm 0.69	2.45 \pm 0.17	17
p (t test)	N S	***	***	N S	N S	N S	N

N S = not significant * $p < 0.05$ *** $p < 0.001$

Serum magnesium

No significant changes were seen in either sex. None of the patients developed hypomagnesaemia (6/18).

Serum creatinine

Serum creatinine decreased but not significantly. Four patients developed an increase over the upper normal limit with a subsequent decrease into the normal range.

Urine investigations

None of the patients developed glucosuria, proteinuria or haematuria during the treatment. One patient had slight proteinuria before the therapy; this disappeared later and an i.v. urography was normal.

Weight

No significant change in weight was found in either sex during 5 months of mefruside therapy though there were considerable intraindividual variations.

Other metabolic parameters

Controls of hemoglobin, ESR and serum sodium showed no significant changes.

DISCUSSION

The value of mefruside as an antihypertensive has been demonstrated in several studies but metabolic effects, especially on plasma lipids, have not been studied earlier in a comparative population of both sexes.

Mefruside induces a significant decrease in serum potassium but the frequency of hypokalaemia after 5 months of therapy seems to be low (2-4%) as no general substitution was given. A surprising finding was that fasting glucose did not increase significantly and none of six glucose tolerance tests in hyperglycaemic patients was pathological. Conn (12) and Morawitz (20) have suggested that a pathological glucose tolerance test during therapy with diuretics should be normalized by means of substitution with potassium but other authors could not verify these findings (27). This study supports the possibility that in addition to exposure to diuretics developed in mature onset diabetes may require general substitution for diabetes.

In this investigation neither cholesterol nor triglycerides increased significantly in contrast to therapy with chlorthalidone and hydrochlorothiazide (1, 2). Seasonal variations in fasting glucose, blood lipids and serum uric acid are well known and can interfere with a comparison.

Creatinine ($\mu\text{mol/l}$)	Weight (kg)
84+13	71.3+13.7
79+17	70.7+14.6
NS	NS
101+11	87.0+9.5
96.17	87.0+8.3
NS	NS

study such as this (15). All the present patients started the treatment during the autumn and completed it during the spring. Serum magnesium did not change significantly during therapy which confirms some results of earlier studies on mefruside (6). Intracellular deposits of magnesium were not studied. Some authors have noted that many patients who developed diabetes mellitus had low magnesium values in the blood but this could be an effect of the development of diabetes (10). The changes in serum magnesium in the present patients were very small and do not correlate with the changes in fasting blood glucose. Serum calcium did not change beyond the normal range in any of the patients. Several studies have shown that serum calcium may increase during treatment with thiazide therapy and in some patients even lead to hypercalcaemia (7, 24). It is suggested in a recent study (11) that the patients who develop hypercalcaemia may have a latent hyperparathyroidism which decreases in plasma albumin during therapy. The result of a significantly lower BP because of the significantly elevated plasma protein level has been demonstrated in middle aged males with an isolated hypertension and is considered partly to be a result of hemoconcentration (25). The conclusion on 25 mg mefruside given daily during

five months to a middle aged population of non alcoholic hypertensive patients results in both supine and standing normotension in the overwhelming majority with few of the metabolic alterations that are normally seen especially in non treated and unselected hypertensive males (4).

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Increased Urinary Protein Excretion after Intravenous Injection of Furosemide in Man

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ACT The furosemide induced increase in excretion, and its relations to 1) the size of molecules as reflected by three enzymes and glomerular filtration rate (GFR), plasma renin activity (PRA) and prostaglandin (PG) E_2 and $F_{2\alpha}$ were studied in 14 outpatients with normal renal function and 13 healthy males. Furosemide (120 mg) was given intravenously, and thereafter the protein excretion and the above parameters were monitored for 1-2 hours. In both groups, furosemide caused a transient increase in protein excretion. The excretion of the largest molecule, β glucuronidase, increased 6.3-fold while those of N acetyl β D glucosaminidase and of the smallest molecule, α amylase, increased by 91 and 37% respectively. GFR increased 100%, but markedly less than the protein excretion. PGE and PGF_{2 α} excretions increased and GFR and changed simultaneously with the excretion of proteins. Furosemide also caused an increase in PRA. This lasted however, much less than the rise in PG and protein excretion. Our results suggest that the furosemide induced increase in protein excretion is 1) related to the size of proteins 2) partly due to the rise in GFR simultaneous with the change in PG excretion. Our findings also agree with the view that furosemide causes changes in glomerular permeability.

Key words: furosemide, protein excretion, glomerular filtration rate, plasma renin activity, prostaglandin excretion.

Acta Med Scand 208 279-1980

It has been found to raise the excretion of protein molecules in urine (6-7-13). Among other determinants transglomerular passage of molecules in general is related molecular weight. In addition to water and electrolyte excretion furosemide increases renal blood flow (8-24). A possible cause of the augmented protein excretion. The increase in renal blood flow has

been attributed at least partly to augmented renal production (25) and urinary excretion of vasodilatory prostaglandins (PG) (12-18-19-23). Moreover furosemide raises plasma renin activity (PRA) (8-14-23) which results in elevated angiotensin II concentration in plasma. This increases the excretion of macromolecules (2). Therefore we decided to study in man 1) the effect of furosemide on the urinary excretion of proteins of various sizes as reflected by three different enzymes and 2) the relations of furosemide induced proteinuria to glomerular filtration rate (GFR), PRA and urinary PG excretion.

METHODS

After an overnight fast and a recumbency of two hours 120 mg of furosemide (Furesis® Laake Turku Finland) was administered intravenously in five minutes in two different groups of subjects. Just before the furosemide injection and during the course of the experiment the subjects drank hourly 200 ml of water. All subjects were voluntary and gave their consent to the study.

Group 1 consisted of 14 outpatients (9 males and 5 females aged 22-50 years) who were examined for various dyspeptic complaints. They had normal renal function and no obvious proteinuria. After the furosemide injection blood samples were taken at 0, 15, 30, 45 and 60 min via an injection needle which was introduced into a cubital vein for the period of the experiment. For the time between samplings the needle was filled with saline. Urine was collected for one hour just before the drug administration and thereafter every 15 min during one hour. The parameters measured were as follows: urine excretion rate, creatinine clearance, unabsorbed fraction of filtered Na⁺, total excretion of proteins and the excretion of three enzymes (β glucuronidase, N acetyl β D glucosaminidase (NAG) and α amylase) the molecular weights of

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Abbreviations: GFR=glomerular filtration rate PG=prostaglandin PRA=plasma renin activity NAG=N acetyl β D-glucosaminidase Na⁺=sodium

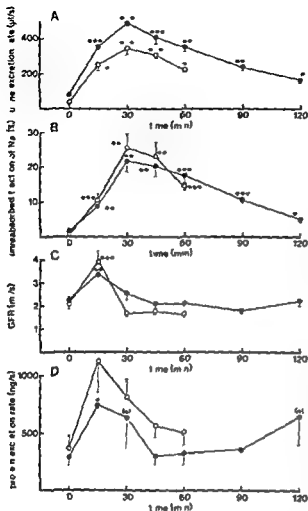


Fig. 1 Effect of furosemide (170 mg i.v.) on the mean (\pm S.E.) urinary excretion rate (A) unreabsorbed fraction of filtered Na⁺ (B) GFR (C) and protein excretion rate (D) in 14 patients with normal renal function (\bullet — \bullet group 1) and 13 healthy males (\circ — \circ group 2). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the values just before furosemide injection (0-values).

which are 218 000, 160 000 and 55 000 respectively) GFR was estimated as endogenous creatinine clearance.

Group 2 consisted of 13 healthy males (aged 20–27 years). After the furosemide injection blood samples and urine were collected every 15 or 30 min for two hours as in group 1. PRA and the above parameters except for the three enzymes were measured in all subjects. In five of them (subgroup 2A) $\text{PGF}_{2\alpha}$ excretion in urine was also measured. In the seven other subjects (subgroup 2B) the effects of a PG synthetase inhibitor on the furosemide induced proteinuria and the alterations in PRA and PGE excretion were studied. That is why the seven subjects in subgroup 2B underwent twice the furosemide treatment in a cross-over manner with and without indomethacin premedication (50 mg orally two hours before the furosemide

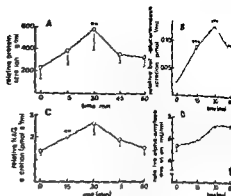
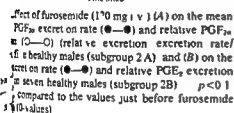


Fig. 2 Effect of furosemide (170 mg i.v.) on the (\pm S.E.) relative protein (A) β -glucuronidase (B) and α -amylase (D) excretion (relative excretion rate/GFR) in 14 patients with normal renal function (group 1) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the values just before furosemide injection (0-values).

injection Indintra[®] Medica Helsinki Finland) interval between the trials was at least seven days.

For estimation of PRA blood samples were drawn into cooled test tubes containing 0.1 N Na EDTA. Within the samples were taken into dried test tubes (for determination of $\text{PGF}_{2\alpha}$ as 31% alcohol soluble plasma or serum samples were stored at -80°C until analyzed). The activities of β -glucuronidase and α -amylase were measured from urine samples at -4°C . Total protein was determined from TC40 rate using Folin-Ciocalteu reagent (modified and Lowry et al. (10)). Creatinine in serum and urine measured by the method based on the Jaffé reaction. The activity of β -glucuronidase was measured by the method of Talalay et al. (20). It is based on the colorimetric determination of phenolphthalein β -D-glucuronide by the action of β -glucuronidase. The activity was determined using p-nitrophenyl- β -D-glucuronide as substrate. The released p-nitrophenol was measured by its absorbance at 420 nm as a method of Banerjee & Basu (11). Alpha amylase was tested with Phadebas amylase kit where the amount of the measuring enzyme in a starch solution was determined by Phadebas (Pharmacia Diagnostics, Uppsala, Sweden). PRA was determined by radioimmunoassay of $\text{PGF}_{2\alpha}$ released in incubation according to the method of Fyhrquist et al. (4). PGs were extracted by the method of Unger et al. (17) and measured radioimmunoassay (9). To separate the free and bound haptens, the antigen-antibody complex was incubated with a large amount of rabbit gammaglobulin and polyethylene glycol was added. Recoveries were more than 80%. PGE_1 The cross reactivity of the $\text{PGF}_{2\alpha}$ antiserum (Cal Assays, Cambridge, Mass.) was less than 0.1%. PGE_2 PGE₁ and PGE₂ at the 50% binding level. The reactivity of PGE₁ antiserum (Sterant Research, Bantam, England) was as follows: PGE₁ 100% PGE₂



Standard errors (S.E.) were calculated and statistical significance between the means was tested by paired *t* test. Regression equations were calculated by the method of least squares.

RESULTS

Groups furosemide increased the mean urine excretion rate by 6-10-fold and the unrecrystallized fraction of Na from 0.6-1.7 to 21.7-25.5% (creatinine clearance) rose by 51-89% and mean excretion rate by 155-205%. The rise in urine excretion rate occurred simultaneously with a rise in GFR but it lasted somewhat longer than that of GFR. The effects of furosemide on these parameters are seen in Fig. 1. In Group I the mean protein excretion rate/GFR (protein excretion) increased by 152% after the intravenous injection (Fig. 2). Beta glucuronidase, the highest molecular weight among the enzymes studied, had a 6.3 fold increase in its relative excretion. The relative excretion of NAG rose by 4.4% and that of the smallest enzyme by 37%.

In group 2 the mean relative protein excretion increased by 112 and 124% ($p < 0.1$) respectively in urine fractions collected 0–15 and 15–30 min after the furosemide injection. $\text{PGF}_{2\alpha}$ (subgroup 2A) and PGE_2 (subgroup 2B) excretion rates increased simultaneously with GFR and the protein excretion rates (Fig. 3, Fig. 1C and D). The excretion of PGE_2 increased more markedly than that of $\text{PGF}_{2\alpha}$. PG and protein excretions remained at the increased level for a longer time than GFR. The relative excretion of PGs (excretion rate/GFR) had also increased significantly and was maximal in the same urine fractions as those of proteins and enzymes (Fig. 2). Within neither subgroup did the protein excretion rates however correlate significantly with the $\text{PGF}_{2\alpha}$ and PGE_2 excretion rates. After the furosemide injection PRA showed a pronounced and persistent rise (Fig. 4). Within group 2 the changes in PRA did not correlate at all with the alterations in GFR and absolute or relative protein excretions.

In subgroup 2B a single oral pretreatment dose of indomethacin (50 mg) did not inhibit markedly the furosemide induced increase in PGE₂. Neither did it modify protein excretion in urine fractions collected before or after the furosemide injection. Indomethacin did not suppress significantly the increase in GFR and urine excretion rate either. It antagonized however effectively the furosemide induced increase in PRA (Fig. 4).

DISCUSSION

Furosemide in a dose of 120 mg i.v. increased markedly the total excretion of proteins and the ex-

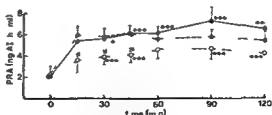


Fig 4 Effect of furosemide (170 mg i.v.) on the mean (\pm SE) PRA in 13 healthy males (●—● group 2) = PRA in seven subjects (subgroup 2B) who underwent furosemide* treatment with (○) and without (●) indomethacin premedication (50 mg orally two hours before furosemide injection) in a cross-over manner * $p < 0.05$ † $p < 0.001$ compared to the values just before furosemide injection (0-values) $p < 0.05$ ‡ $p < 0.01$ compared to the means without indomethacin premedication

cretion of three enzymes with different molecular weights in urine. The excretion of β glucuronidase with the largest molecular size increased most and that of the smallest molecule α amylase rose least. This agrees with our previous findings that the furosemide induced increase in macromolecule excretion is related to molecular size (13).

The increase in protein excretion caused by furosemide has been found to correlate with the rise in GFR (15). In our study the protein excretion rate also increased simultaneously with GFR. The rise in GFR can however only partly explain the increased protein excretion rate since the relative protein excretion (excretion rate/GFR) was also elevated and maximal in the 15–30-minute urine fraction when GFR had already returned to the initial level. As discussed elsewhere (13) the possible inhibition by furosemide in tubular protein reabsorption seems neither to explain the furosemide induced proteinuria. In that study the excretion of high molecular weight proteins was shown to have increased significantly compared with that of low molecular weight proteins which are the ones that should be easily influenced by changes in tubular handling. In addition in the present study the rise in protein excretion was of a shorter duration than the inhibition of Na^+ reabsorption or the enhancement of the urinary excretion rate. NAG and β glucuronidase have been shown to originate also from the renal tissue itself especially in connection with renal injuries (5, 11, 16). In this study the tubular origin of the increased enzyme excretion after furosemide cannot be excluded but a much more marked rise in the excretion of large molecules than in that of small ones counteracts the idea that the increase in enzymuria results exclusively from tubular cells. Thus the current findings agree with the view that furosemide increases the glomerular permeability for serum proteins.

Furosemide increases renal blood flow (8, 24, 25) a possible cause of the rise in GFR. This increase in blood flow has been found to be associated with the augmented excretion of vasodilatory PGs (12, 18, 19, 23). In this study furosemide raised the excretion of both the vasodilatory PGE_2 and the vasoconstrictive $\text{PGF}_{2\alpha}$ but the excretion of PGE_2 increased more markedly than that of $\text{PGF}_{2\alpha}$. PG excretions remained however elevated for a longer time than GFR. The increase in GFR if mediated by vasodilatory PGs could thus be antagonized by some unknown factor(s) activated

e.g. by the intrarenal production of the PGs themselves. The renin-angiotensin system is one of the possible candidates. It is known to be under the influence of PGs and to be activated by them (17). In our study PRA actually increased simultaneously with the excretion of PGs and GFR showed a persistent rise until the end of the 1-week period while PGs had returned to the initial level.

Indomethacin in a dose of 50 mg three daily has recently been reported to decrease protein excretion in patients with proteinuria. It has been suggested to be associated with changes in renal haemodynamics by indomethacin as a result of the decrease in PRA and PG synthesis (18). In our subjects PG excretion but not PRA exhibited an alteration pattern similar to the protein and enzyme excretion after the furosemide infusion. Thus we may propose that furosemide induced protein excretion is closely associated with PG production but not with PRA. The proposal is further supported by our finding that the indomethacin dose which was incapable of suppressing PG excretion essentially inhibited the rise in protein excretion but did not modify the protein excretion in the late phase of the study. The lack of correlation between the changes in PG and protein excretions of individual subjects with the experimental results does not however fit well with this assumption.

In conclusion intravenously administered furosemide increases transiently the urinary protein excretion which is related to the molecular size of proteins. The changes in protein excretion occur simultaneously with the change in PG excretion and are partly due to the changes in GFR. These findings also agree with the view that furosemide increases the permeability of glomerular capillaries for macromolecules.

ACKNOWLEDGEMENT

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Psychological and Social Problems Encountered in Active Treatment of Chronic Uraemia

III Prediction of the Living Donor's Psychological Reaction

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ACT Sixteen kidney donors were interviewed by a psychiatrist and given the Rorschach test and one year after operation. We used our study as a basis in predicting the expectable psychic traumatization that the donation would provoke. In 11 of the donors the prediction was correct. A trend towards a better ability was observed in cases of successful donation. An unsuccessful operation is always followed by psychological complications reducing the possibilities of an accurate prediction and necessitating psychotherapeutic help. The present procedure is clinically simple and requires relatively little

and psychiatric methods as well, which here also comprised a psychiatric interview and the Rorschach test.

The psychiatric interview lasted regularly for about 45 min. This time was divided between two objectives: 1) to obtain a fairly accurate global overview on the prospective donor's personality, and 2) to assess the important factors concentrating around the donation procedure as such. The latter part of the interview was based on previous experience and our earlier studies. One year after the transplantation the donor was seen again. This time the 45-minute psychiatric interview was dedicated mainly to the examination of the phenomenology of the donor's experiences during the transplantation procedures and the time elapsed after donation.

The psychiatric interview was on both occasions supplemented by the Rorschach test. This test, one of the most established standard procedures in clinical psychology, is well suited to unearthing less conscious intrapsychic processes and thus giving a more deep and broad description of the examinee's psyche when used together with interview data.

During the examination period 34 prospective donors were examined for transplantation. Sixteen of them became kidney donors because their histocompatibility proved acceptable and none of the other somatic contraindications were demonstrable. Of these 4 gave their kidney to their child, 10 to their brother or sister, one to her grandchild and one to his aunt. Transplantation was unsuccessful in four cases. One of these recipients died within one month after transplantation. On the basis of the examination before transplantation, a brief clinical vignette for each of the 16 donors was worked out by a team concentrating especially on the psychodynamic factors which were found significant in our previous report (1). These vignettes were then crystallized into a prediction of how traumatically the donation would be experienced by the donor. In accord with our previous report, the predictions were classified as 1) no trauma, 2) mild trauma, 3) moderate to severe trauma (Table I).

Each donor was re-examined as accurately as possible one year after the donation, as feasible, using the same methods as before donation. As previously, the team held a conference on each case to assess how traumatic the donation had been.

Acta Med Scand 208: 285-287 1980

the long-term psychological consequences of donation. 64 volunteer donors were subjected to a psychiatric interview and given the Rorschach test at 6 months to 6 years after transplantation. Special attention was paid to how the donation process had been experienced and how it reflected in the donor's personality (1). This study revealed that donation may in some cases have a negative influence on the donor's ability. In another study on 111 volunteer donors examined before and one year after donation, we conducted our purpose was to clarify to what extent it is possible to predict the donor's reaction to the donation, i.e. how dramatic the reaction of the transplantation would be.

SUBJECTS AND METHODS

purpose. Every prospective donor in the years 1968-1978 was systematically examined by psychological

Skeletal Scintigraphy with Technetium Diphosphate in Multiple Myeloma— a Comparison with Skeletal X-Ray

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ACT Twenty one patients with multiple myeloma were examined in close time relation with X ray survey and bone scanning using ^{99m}Tc diphosphate. Results indicate that X-ray superior to bone scan in detecting myelomatous lesions. Approximately twice as many lesions detected by X ray as by bone scan. An exception: general finding is the lumbar spine and ribs in which the two methods are equally reliable. A negative bone scan does not exclude the possibility of a myelomatous bone lesion.

Key words: bone scanning, skeletal scintigraphy, skeletal X-ray, multiple myeloma.

Acta Med Scand 208 289 1980

Skeletal lesions are important manifestations of multiple myeloma. The mechanism behind bone destruction is unknown but has been ascribed to a direct effect of the neoplastic bone marrow cells (1). Plain X-ray of the skeleton has for years been a routine procedure in the search for bone lesions (13). Radionuclide bone scanning with various technetium isotopes has recently gained widespread use with the purpose of detecting bone metastases from various malignant tumors (1, 15, 18). Many reports have indicated that skeletal scintigraphy in this respect is simpler and more sensitive than plain skeletal X-ray (2, 4). Skeletal scintigraphy has been used to detect bone lesions also in multiple myeloma but results have been contradictory and seem to indicate that scintigraphy is less effective in this disease than skeletal X-ray (2, 7, 16).

Therefore, it is important to perform a comparative study of the skeletal X-ray and scintigrams in a series of multiple myeloma patients who had been examined in close relation.

PATIENTS AND METHODS

Twenty-one patients with multiple myeloma were studied. Diagnostic criteria included numerous lytic bone lesions, bone marrow findings with marked increase in plasma cells with malignant characteristics (chromophobe nucleoli, multinucleated cells) and tendency to sheet like growth, plasma IgG or IgA M-component and/or urinary M-component. Thus, two patients had urinary M-components only and together with plasma hypogammaglobulinemia fulfilled the criteria for a diagnosis of light chain disease (19). One patient lacked M-component in plasma and urine and was classified as having non-secretory myeloma (11). In addition, several patients also had myeloma accompanying features like anemia, hypocalcemia or hypercalcemia.

The study was carried out between Oct. 1977 and Dec. 1978. All patients were admitted to or seen in the Outpatient Section of the Department of Internal Medicine, University Hospital Linköping, Sweden. Skeletal X-ray studies and scintigraphy were performed in the Departments of Radiology and Radiophysics. Skeletal X-ray and scintigraphy were performed at a maximum interval of 30 days. In most instances, however, only a few days elapsed between the two examinations.

Skeletal X-ray survey included the skull, spine (except for the cervical spine), pelvis, arms and legs (hands and feet were not included). Radionuclide bone scanning involved intravenous injection of 15 mCi technetium ethylene hydroxybisphosphate or methylene diphosphate. The entire body of the patient was scanned after 3 hours with a gamma camera (Maxicamera I, General Electric).

For the purpose of comparing the effectiveness of the two methods, 15 regions of the skeleton were defined as follows: 1) Skull, 2) thoracic spine, 3) lumbar spine, 4-5) right and left rib cage, 6-7) right and left humerus, 8-9) right and left lower arm, 10-11) right and left pelvis, 12-13) right and left femur, 14-15) right and left lower leg. The number of "positive" (with one or more lesions) regions detected by the two methods were enumerated and summed up. X-ray films were reviewed by two radiologists independently.

It was registered if a patient had received radiotherapy to a particular region, since it has been reported that radiotherapy may influence the result of skeletal scintigraphy by reducing the uptake of the nuclide (5).

RESULTS

In 17 cases examined at an interval of less than 30 days skeletal X ray demonstrated lesions in 101 of 243 regions studied while skeletal scintigraphy revealed only 54 positive of 243 regions studied. In 4 cases examined at intervals of 2-3 months the figures were 18 positive of 60 regions examined with skeletal X ray and 8 positive of 60 examined with skeletal scintigraphy. Correction for given radiotherapy did not influence these results. Close correlation between results obtained by the two independent X ray reviewers was noted. Thus X ray examination revealed more lytic bone lesions in most regions than scintigraphy. However the two methods seemed to be equally effective in regions involving lumbar spine and rib cage.

DISCUSSION

This study shows that skeletal scintigraphy is inferior to skeletal X ray in detecting bone lesions due to multiple myeloma. Approximately twice as many lesions as by scintigraphy were detected by X ray. An important conclusion is also that a normal skeletal scintigraphy does not exclude myelomatous skeletal lesions evident on X ray.

There seems to be an exception to this general rule. In the lumbar spine and the rib cage the two methods of examination are equally effective. The reason for this is obscure but may be related to increased occurrence of pathological fractures (or infractions) in these weight bearing and vulnerable regions. Fractures and callus formation in themselves cause increased uptake of the isotope and the number of lesions detected by the scintigraphic procedure may be increased (3, 12, 17). The cause of the decreased sensitivity of skeletal scintigraphy in multiple myeloma as compared to bone metastases of various malignant tumours is not clear at present. However the uptake of bone seeking isotopes seems to be related mainly to osteoblastic processes and neoplasms that metastasize to bone are associated with both osteolytic and osteoblastic activity. Multiple myeloma on the other hand is almost always osteolytic with very little new bone formation (13). Also evidence has been presented (14) for the secretion of an osteoclast stimulating factor in myeloma that appears closely related if not identical to osteoclast activating factor that has recently been identified (6, 10). Thus extensive osteoclast activity in myeloma may well suppress

osteoblastic activity and therefore inhibit uptake of bone seeking isotopes.

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100

Urinary Excretion of Inorganic Sulfate, Ester Sulfate, Total Sulfur and Taurine in Cancer Patients

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ABSTRACT The urinary excretion of inorganic sulfur sulfate, taurine and total sulfur has been studied in 11 patients with malignancy, and compared with those of an age-matched reference group. The excretion of inorganic sulfate was significantly lower in the cancer group, whereas an increased excretion of ester sulfate was observed. The excretion of total sulfate (inorganic plus ester sulfate) and increased excretion of "neutral sulfur" (total sulfur minus total sulfate) in the cancer group calculated from these data. If these values were related with respect to creatinine, no significant differences were found for inorganic and total sulfate in the two patient groups, whereas ester sulfate and "neutral sulfur" fractions were still increased in the cancer group. The enlarged "neutral sulfur" could to a certain extent be explained by an increased excretion of taurine. The mechanism behind these findings is discussed.

Key words: inorganic sulfate, neutral sulfur, taurine.

Papadopoulos (10) reported that cancer patients showed a decreased urinary excretion of inorganic and total sulfur and a concomitant increase in the neutral sulfur fraction. She concluded that this was due to oxidation of sulfur containing amino acids and other sulfur compounds found in the body. As no reports confirming or disputing her findings have appeared in the literature, we have conducted the present investigation.

PATIENTS AND METHODS

The study group consisted of 8 men and 10 women (mean age 66 years, range 47-80) all with histologically verified malignancy located in the female genital tract, breast, lung, large bowel, or kidney. One patient had evidence of liver metastases but none had any

clinical signs of malnutrition. Patients treated for minor diseases in the Medical and Orthopedic Departments (7 men and 6 women with a mean age of 66 years (range 55-83)) were used as reference group. All patients were on a standard hospital diet and had no laboratory signs of kidney dysfunction.

All 24 hour urine collections were made with thymol isopropanol as preservative (8). Urine samples were analyzed for total sulfur, inorganic sulfate and ester sulfate according to Lundquist et al. (7). Total sulfate was calculated as the sum of inorganic and ester sulfate and the neutral sulfur fraction as the difference between total sulfur and total sulfate. Taurine was determined according to Sorbo (15). Creatinine analysis was performed according to the standard Jaffe method (2).

RESULTS

The results shown in Table I demonstrate that the excretion of inorganic and total sulfate was decreased but that of ester sulfate somewhat increased in the cancer group. Furthermore a significantly increased excretion of neutral sulfur was observed in the cancer patients.

We have found that a sex difference exists for the excretion of inorganic sulfate and total sulfur in healthy human subjects (7). This difference disappeared when the values were recalculated relative to creatinine. Consequently we recalculated our excretion values for the cancer and reference groups with respect to creatinine excretion. As shown in Table I the differences in excretion of inorganic and total sulfate between the two groups were no longer significant, whereas the ester sulfate and neutral sulfur excretions were still significantly higher in the cancer than in the reference group.

As it is known that certain analgesics are excreted as their sulfate esters (6) we checked that our cancer patients did not receive such compounds. The increased ester sulfate excretion was thus unexplained. Similarly certain sulfur-contain-

Table I Urinary excretion of sulfur metabolites in cancer patients (mean \pm S.D.)

Compound	Cancer group		Reference group	
	mmol/24 h	mol S/mol creatinine	mmol/24 h	mol S/mol creatinine
Total sulfur	16.1 \pm 7.6	2.06 \pm 0.67	19.3 \pm 5.0	1.86 \pm 0.29
Inorganic sulfate	11.0 \pm 6.0*	1.36 \pm 0.48	15.7 \pm 4.1	1.52 \pm 0.28
Ester sulfate	2.30 \pm 0.95*	0.32 \pm 0.17*	1.16 \pm 0.69	0.16 \pm 0.05
Total sulfate	13.2 \pm 6.5*	1.68 \pm 0.56	17.6 \pm 4.6	1.72 \pm 0.25
Neutral sulfur	3.06 \pm 1.8*	0.39 \pm 0.17*	1.72 \pm 0.86	0.17 \pm 0.07
Taurine	1.45 \pm 0.33*	0.19 \pm 0.062*	0.50 \pm 0.23	0.047 \pm 0.017

* Significantly different from the reference group Student's *t* test ($p < 0.05$)

ing antibiotics such as penicillin and cephalosporin can be expected to increase the "neutral sulfur" fraction. However, none of our cancer patients received such compounds. An important component of the neutral sulfur fraction is taurine (9). The determination of taurine showed that the excretion was significantly higher in cancer patients than in the reference group (Table I). This taurinuria explained at least to a large extent the increased excretion of "neutral sulfur" in cancer patients.

DISCUSSION

Our results that the absolute amounts of inorganic sulfate found in the urine of cancer patients were lower than the excretion in the control group agree with the results of Papadopoulou (10). However, her conclusion that cancer patients have an impaired ability to oxidize sulfur amino acids to sulfate appears unlikely as we found that the excretion of inorganic sulfate was not significantly different from that of the cancer patients when related to creatinine excretion. It should be noted that all our patients had normal serum creatinine values in accordance with a normal kidney function.

We found an increased excretion of ester sulfate in the group of cancer patients. The same conclusion could be drawn from the data presented by Papadopoulou (10). Raised amounts of ester sulfate appear in the urine from an increased production of phenols by bacterial degradation of aromatic amino acids (tryptophan, tyrosine, and phenylalanine) in the bowels (1). By this mechanism patients suffering from constipation show increased excretion of ester sulfate (3). Obstipation cannot be excluded as a cause of the increased excretion of ester sulfate in our cancer patient group.

A noteworthy finding was an increased taurine

excretion in the cancer group. Taurine is one of the most abundant amino acids normally found in the body. Taurine is synthesized from cysteine in the liver and possibly other tissues (3) and is mainly located to the intracellular compartment. A high concentration of taurine is found in many tissues especially in striated muscle, brain, thrombocytes, and leukocytes. In some of these tissues the concentration of taurine is 10 times higher than any other amino acid. Taurine is mainly eliminated via the kidney; thus, the daily excretion represents only a few per cent of the total body taurine (17). One important function of taurine is bile acid conjugation. In the liver the bile acids are conjugated with taurine, thus increasing evidence that taurine is functionally a neurotransmitter or neuromodulator in the brain. Its role in the retina has been presented recently (18). Furthermore, taurine plays a role in the regulation of membrane excitability in the heart and the increased ventricular concentration of taurine is a congestive heart failure (4).

Taurinuria observed in our cancer patients has, to our knowledge, not been reported in the literature. An increased taurine excretion occurs after whole body irradiation (5) and cytostatics (13) but not in our patients receiving such treatment. As high concentrations of taurine are present in many tissues, cell damage will induce an increased taurine concentration in plasma and, as that taurine has a high renal clearance (16), it is rapidly excreted in the urine. A number of pathological conditions of cellular damage have been reported to cause taurinuria (5, 11). Consequently, the increased taurine excretion found in cancer patients cannot be regarded as a specific sign of cancer but as a non-specific response to cellular damage. It should be noted that the taurinuria observed by us was of the same order of magnitude as the increased excretion of cancer studied. The number of patients

small and the results should be interpreted cautiously

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2

Blood Glucose Control and Lipolysis in Diabetes Mellitus

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ABSTRACT The relationship between the blood glucose level and the rate of lipolysis was investigated in 10 maturity onset diabetics (MOD) and 9 juvenile diabetics (JOD). Subcutaneous adipose tissue biopsies were taken with and without isoprenaline or noradrenaline. Before antidiabetic treatment the blood glucose concentration was positively correlated with the catecholamine-induced rates of lipolysis in MOD ($r=0.41-0.60$) and in JOD ($r=0.51-0.71$). The reduction in blood glucose concentration during antidiabetic treatment was positively correlated with the reduction in the rate of lipolysis in MOD ($r=0.40-0.64$) and in JOD ($r=0.75-0.90$). Fat cell diameter and blood glucose level were not correlated either before or after treatment in diabetics. In diabetes mellitus blood glucose homeostasis and rate of lipolysis in subcutaneous adipose tissue seem to be associated. The relation is most apparent in JOD.

Key words: diabetes mellitus, adipose tissue, lipolysis.
Acta Med Scand 208: 297-298, 1980.

An accelerated rate of lipolysis in adipose tissue in diabetes mellitus (2-5, 11, 12) is of considerable clinical significance since it may be a contributory factor to several complications of diabetes mellitus. The purpose of the present study was to ascertain whether in diabetes mellitus the rate of lipolysis is related to the blood glucose control. Basal and catecholamine induced glycerol release in vitro was determined in fragments of subcutaneous adipose tissue from untreated patients with maturity onset (MOD) and juvenile onset (JOD) diabetes. The effect of antidiabetic treatment on lipolysis and blood glucose was also investigated.

STUDY POPULATION

The study consisted of inpatients with recently diagnosed diabetes. 9 with JOD and 19 with MOD. They were

otherwise healthy and no selection on the basis of sex was made. A subcutaneous fat biopsy was taken from the femoral region after an overnight fast. At this time no antidiabetic treatment had been received. Twenty-one patients were re-examined during antidiabetic therapy for at least 3 months. This consisted of diet alone or administration of sulphonylurea in MOD (16 patients) and insulin in JOD (5 patients). The patients were re-admitted to hospital and subcutaneous fat specimens were taken from the contralateral region after an overnight fast before administration of insulin or sulphonylurea. At this time four JOD patients and one MOD patient had glucosuria (less than 200 mmol/24 h). None had ketonuria.

The investigation was approved by the Ethical Committee of the Karolinska Institute. Details of the study were explained to the patients and their consent was obtained.

METHODS

Local anaesthesia was induced with prilocaine chloride as described previously (2). The blood glucose concentration was measured (7) on 3 or 4 occasions during 12 hours before and after the biopsy. A mean blood glucose concentration was calculated from these values and the fasting mg value.

Adipose tissue was divided into 50 mg segments and preincubated for 30 min in Krebs Henseleit bicarbonate buffer (37°C, pH 7.4) containing 40 mg/ml of dialyzed bovine serum albumin (Armour Fraction V, England). Then adipose tissue (100 mg) was incubated for 2 hours in 1 ml of fresh buffer of the same type and two aliquots of the medium were removed for glycerol estimation (6). All incubations were done in triplicate or quadruplicate with air as the gas phase.

The fat cell diameter was determined by the method of Sjostrom et al. (13). Fat cell volume and fat cell number were calculated by the method of Hirsch and Gallian (9).

Noradrenaline bitartrate (Astra, Sweden) and isoprenaline hydrochloride (Winthrop, England) were added to

Abbreviations: MOD = maturity onset diabetes (diabetics); JOD = juvenile onset diabetes (diabetics).

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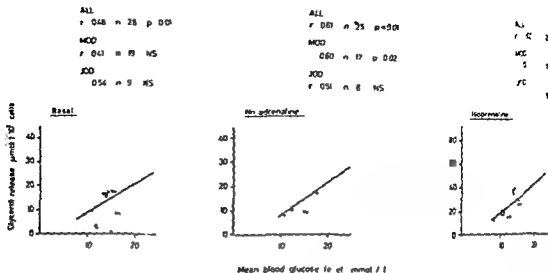


Fig 1 Correlation between mean blood glucose level and rate of lipolysis in untreated diabetics. Adipose tissue was incubated in the absence and presence of noradrenaline or isoprenaline. The mean blood glucose level was deter-

mined and compared with the rate of glycerol release *in vitro* using linear regression analysis ($y = ax + b$). MOD: The regression line represents the whole w-

the medium *in vitro* to give a final concentration of 6 $\mu\text{mol/l}$, the level known to furnish the maximum effect. (3) Linear regression was examined by the method of least squares.

RESULTS

In the whole material before treatment for diabetes mellitus there was a significant positive correlation between the mean blood glucose concentration and the rate of glycerol release *in vitro*, both in the

presence and absence of catecholamines. When the patient group was divided into MOD, the values for the correlation coefficient were in general higher in JOD (0.51–0.71) than in MOD (0.41–0.60). The fasting blood glucose and the rate of lipolysis were also significantly related in the whole patient series (data not shown).

In the patients who were re-examined after antidiabetic therapy, the decrease in fasting blood glucose concentration was positively and significantly

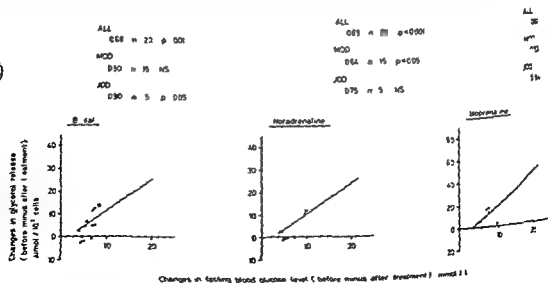


Fig 2 Influence of antidiabetic therapy on the relationship between fasting blood glucose level and rate of lipolysis. For further details see legend to Fig 1.

ed with the decrease in the basal and
 Jamine stimulated rates of lipolysis (Fig 2)
 values for the correlation coefficients were
 d in JOD ($r=0.75-0.90$) than in MOD
 -0.64). In the whole material the decrease
 blood glucose level was also significantly
 ed with the decrease in the rate of lipolysis
 (shown)
 * was no correlation between fat cell size
 od glucose concentration

DISCUSSION

obilization from the fat depots is known to
 anced in JOD and MOD (3-5 8 12). Al
 it decreases during antidiabetic therapy (4)
 is been found both in vivo (11) and in vitro
 lipolysis is enhanced also when the disease
 n brought under control. This suggests that
 ns in lipolysis and glucose homeostasis are
 ocated in diabetes mellitus. In the present
 however there was a positive correlation
 the plasma glucose level and the lipolytic
 the adipose tissue before treatment as well
 catecholamine-stimulated as for unstimulated
 lipolysis. Furthermore when hyperglyc
 was brought under control the reduction
 blood glucose level and the decrease in the
 lipolysis were correlated. This is observed
 JOD and MOD but the relationship appears
 rmer in JOD. The correlation between the
 glucose level and the rate of lipolysis strongly
 s that the homeostatic mechanisms that
 glucose utilization and free fatty acid mobili
 in diabetes mellitus are interrelated at least

mechanisms behind the link between blood
 control and lipolysis are not known. The
 nous insulin level or different degrees of in
 sistance are probably not the main factors
 sible for the relationship. If so one would
 a better correlation in MOD than in JOD. If
 the opposite phenomenon was observed
 present study. Neither is it probable that the
 ship is solely conditioned by the action of
 nous catecholamines since maximal effec
 ncentrations of catecholamines were used in
 has also been suggested that the fat cell size

is a factor in the control of metabolism in diabetes
 (10). In the present investigation however fat cell
 size was not correlated with the blood glucose level.

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Comparative Single-Dose Kinetics and Effects of Four Sulfonylureas in Healthy Volunteers

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ACT The single dose kinetics and effects of tolbutamide (400 mg) chlorpropamide (250 mg), tolbutamide (5 mg) and glipizide (5 mg) were compared in 17 healthy male volunteers by measurements of concentrations of the drugs and of plasma and blood glucose. The drugs were administered on an empty stomach and together with a standardized breakfast. The concentrations of tolbutamide and chlorpropamide were measured by gas chromatography, those of glipizide with high pressure liquid chromatography, those of glibenclamide by radioimmunoassay and those of glipizide by the hexokinase method. Glipizide and glibenclamide were more potent inducers of insulin than tolbutamide and chlorpropamide. As the concentrations of the two drugs were in the range of nmol/l and the latter two in the $\mu\text{mol/l}$ range the findings support the notion that the intrinsic activity of a second-generation sulfonylurea is at least as great as that of the two first generations. Glipizide seemed to be a more potent and rapid insulin releaser than glibenclamide but the difference was secondary to biopharmaceutical differences in the two preparations. The bioavailability of glipizide was apparently greater than that of glibenclamide. Both glibenclamide ($t_{1/2} = 1.8$ h) and glipizide ($t_{1/2} = 4.3$ h) showed much shorter elimination half-lives than tolbutamide (7 h) and chlorpropamide (34 h). It seems probable, however, that all these are not fully informative as to the time of action of the drugs.

Key words: sulfonylureas kinetics effects
Acta Med Scand 208 301 1980

A 10-year follow up indicates that impaired glucose tolerance if left untreated carries a great risk of development into manifest diabetes but that development can be prevented or postponed by strict regulation of diet and tolbutamide

(12). Another analysis of the same material has shown that this medication reduces rather than enhances cardiovascular morbidity (8). These findings suggest that sulfonylurea drugs are beneficial rather than harmful and this has reawakened interest in the search for an optimal sulfonylurea preparation.

The aim of sulfonylurea treatment is normalization of glucose economy. In particular it is important to enhance the disposition of the large carbohydrate loads that follow each meal. On the other hand the sulfonylurea effect should not prevail between meals and the drug should not be liable to provoke long lasting hypoglycemia. Accordingly the sulfonylurea should be as potent, rapid acting and short acting as possible. In an attempt to compare these capacities of tolbutamide, chlorpropamide, glibenclamide and glipizide, single standard doses of these drugs were given both with and without a standardized breakfast in seven healthy volunteers. The effects on plasma insulin and blood glucose were recorded together with the concentration profiles of each drug.

SUBJECTS

Seven male volunteers participated in the study. They were considered healthy as judged by conventional physical examinations and routine blood tests. Their mean ages and ideal body weights were 26.2 (\pm S.D. 1.9) years (range 23.5-29) and 96.8 (\pm S.D. 5.0) kg (range 90.1-101.4). Each subject was extensively informed and gave written consent.

METHODS

Protocol

On 9 different mornings at intervals of at least one week the subjects came to the laboratory after a 10 h fast (10

Abbreviations: $t_{1/2}$ = half life; AUC = area under the curve

p m -8 a m). All underwent each of the following treatments: 1) A standardized breakfast meal without drug intake 2-5) meal plus 500 mg tolbutamide (Artosin® Boehringer Mannheim Mannheim Federal Republic of Germany) 250 mg chlorpropamide (Diabinese® Pfizer Groton Conn USA) 5 mg glibenclamide (Daonil® Hoechst Frankfurt Federal Republic of Germany) or 5 mg glipizide (Min(i)diab® Carlo Erba Milan Italy) 6-9) the same drugs without meal (fasting continued for another 4 h). The standardized meal consisted of 400 ml low fat milk 20 g white bread with 5 g butter and 35 g cheese and 150 ml non sweetened black coffee yielding a total energy of 430 kcal or 1 800 kJ.

Analyses

Serial blood samples were drawn via an indwelling antebrachial venous polyethylene catheter. Blood glucose and plasma insulin were followed for 3 h. Blood glucose concentrations were determined by the hexokinase method (4) and plasma insulin concentrations were measured by a commercial solid phase radioimmunoassay (Phadebas Insulin Test® Pharmacia Uppsala Sweden). The serum concentrations of the drugs were monitored for 24-48 h. Tolbutamide and chlorpropamide were determined by gas chromatography (11). The possibility of chromatographic pyrolysis of sulfonylurea was avoided by using an all glass system and a low injection temperature. The interassay variability was 5.1% (SD of a serum pool value). Glipizide was measured by a high pressure liquid chromatographic assay with a sensitivity of 20 nmol/l and an interassay variability of 6.2% (15). Glibenclamide was determined by radioimmunoassay (7). The antibody reacts not only with glibenclamide but also with its hydroxylated active metabolite. However, as the metabolite is normally present only in minute amounts and has a very short half life ($t_{1/2}$) (14) its interference would be negligible in a single-dose study on healthy volunteers. The assay sensitivity was 20 nmol/l and the interassay variation 9.9%.

Calculations

The serum concentrations of the four drugs were plotted against time. Peak concentration was defined as the highest value recorded. As the absorption and elimination phases could not be safely distinguished from each other no calculations were carried out as to absorption kinetics. The elimination $t_{1/2}$ was calculated by regression analysis. The area under the curve (AUC) was estimated by the trapezoidal rule. The statistical significance of differences was calculated by Student's paired t tests on intrasubject data pairs and by Wilcoxon's signed rank test.

RESULTS

Drug concentrations in serum

Fig 1 shows the single dose kinetics of tolbutamide (500 mg) chlorpropamide (250 mg) glibenclamide (5 mg) and glipizide (5 mg) ingested in the fasting state. The kinetic data under fasting and non fasting conditions are given in Table 1. It can be seen that the former two drugs yielded serum concentrations

of several $\mu\text{mol/l}$ while those of the latter two in the nmol/l range. The elimination of propamide (mean of 34 h in the fasting state) was much slower than that of tolbutamide ($t_{1/2}$ 0.001) which in turn was slower ($p < 0.01$) than that of glipizide (4.3 h) and glibenclamide (1.8 h), the shortest of all (1.8 h $p < 0.05$ vs glipizide). $t_{1/2}$ figures were recorded when the drugs were in the non fasting state (Table 1).

Both in the fasting and non fasting state, the mean peak concentration of glipizide was slightly but not significantly higher than glibenclamide. A significant difference was recorded with respect to the time to reach peak concentration in the fasting state: the mean t_{max} for glipizide while that of glibenclamide was 1.5 h ($p < 0.05$). The mean AUC of glibenclamide was smaller than that of glipizide both in the fasting and non fasting state; the latter but not the former difference reached statistical significance ($p < 0.05$).

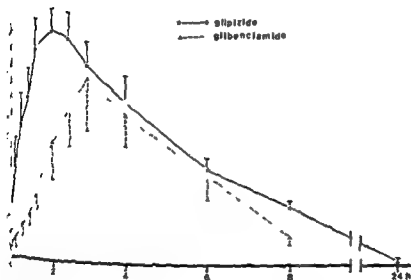
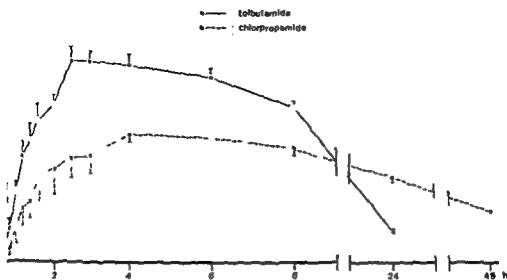
Plasma insulin

Fig 2 demonstrates the plasma insulin profile following intake of the four drugs in the fasting state. The pre-exposure mean values did not differ significantly. Glipizide promoted an increase in insulin that was significantly ($p < 0.05$) above control at 45, 60 and 75 min after drug intake. Increments seen after administration of glibenclamide and tolbutamide did not reach statistical significance. Chlorpropamide evoked little change at all.

Table II shows the plasma insulin levels following intake of the four drugs both with and without standardized breakfast. The pre-exposure values did not differ significantly. Both glipizide and glibenclamide yielded greater plasma insulin increments than did the meal only. However, statistical significance was reached only for glibenclamide at 90, 120 and 150 min ($p < 0.05$). No significant difference was recorded at any interval between plasma insulin concentrations following meal plus meal and glibenclamide plus meal.

Blood glucose

The blood glucose profiles following intake of the four drugs in the fasting state are shown in Fig 3. Significant reductions ($p < 0.05$) of the blood glucose concentrations were recorded at 45 and 60 min (tolbutamide and glipizide) at 75, 90 and 120 min (tolbutamide, glipizide and glibenclamide).



Single dose kinetics of (a) tolbutamide (500 mg) and chlorpropamide (250 mg) and (b) glipizide (5 mg) and glibenclamide (5 mg) in seven healthy volunteers following intake on an empty stomach. The calculated kinetic data are given in Table I

in (glipizide and glibenclamide). The mean blood glucose reduction after chlorpropamide did not reach statistical significance at any interval studied. Glipizide seemed to evoke a more rapid glucose diminution than glibenclamide but this difference did not reach statistical significance at any interval studied. Both these drugs on the other hand evoked glucose reductions that were significantly ($p < 0.05$) more marked than those following tolbutamide at 60, 75, 90 (glipizide), 120 and 180 min (glipizide and glibenclamide).

Concurrent administration of drugs and meal (Table III) yielded smaller blood glucose incre-

Table I Pharmacokinetic parameters of four sulfonylureas in seven healthy volunteers following oral dose ingested both on an empty stomach and with a standardized breakfast (mean \pm S.E. in parentheses)

C_{max} = peak concentration t_{max} = time to peak concentration

	Tolbutamide (500 mg)	Chlorpropamide (250 mg)	Glibenclamide (5 mg)	Glipizide (5 mg)
<i>Fasting</i>				
C_{max} (μ mol/l)	153 \pm 8 (122-178)	100 \pm 4 (87-112)	0.592 \pm 0.129 (0.231-1.182)	0.703 \pm 0.076 (0.346-0.916)
t_{max} (h)	3.4 \pm 0.3 (2.5-4.1)	3.2 \pm 0.6 (1.5-5.9)	3.4 \pm 0.5 (2.5-6)	2.1 \pm 0.2 (1.5-2.9)
$t_{1/2}$ (h)	7.2 \pm 0.3 (6.1-8.0)	34 \pm 3 (21-43)	1.8 \pm 0.3 (1.1-2.8)	4.3 \pm 0.7 (2.5-7.3)
AUC (μ mol \times h \times l ⁻¹)	1976 \pm 76 ^a (1587 \pm 2180)	2806 \pm 157 ^b (2121 \pm 3317)	2.109 \pm 0.411 ^c (0.706-3.727)	2.963 \pm 0.3 (1.764-4.385)
<i>Non fasting</i>				
C_{max} (μ mol/l)	140 \pm 9 (122-192)	86 \pm 5 (65-101)	0.591 \pm 0.049 (0.453-0.868)	0.717 \pm 0.049 (0.496-0.834)
t_{max} (h)	4.0 \pm 0.8 (1.5-7.9)	5.4 \pm 0.8 (1.3-8.2)	2.6 \pm 0.3 (2-4)	2.4 \pm 0.2 (1.5-3.0)
$t_{1/2}$ (h)	7.0 \pm 0.3 (5.4-7.9)	40 \pm 4 (26-56)	1.6 \pm 0.1 (1.2-1.8)	3.9 \pm 0.6 (2.0-5.7)
AUC (μ mol \times h \times l ⁻¹)	1825 \pm 77 (1571-2152)	2728 \pm 190 ^b (1997-3295)	2.261 \pm 0.233 (1.920-2.299)	2.957 \pm 0.111 ^c (2.560-3.341)

^a 0-24 h ^b 0-48 h ^c 0-8 h

ments than did the meal only. Significance ($p < 0.05$) was reached both with glipizide (at 75-180 min) and glibenclamide (at 150 min).

DISCUSSION

The kinetic characteristics of sulfonylureas have been described previously (1-16) and the observa-

tions made in the present study are in agreement therewith. However there are few studies concerning the comparison and this was the major concern of the investigation. Particular emphasis was placed on differences in intrinsic activity and in rapidity of onset. The drug measurements showed that

Table II Plasma insulin levels in seven healthy volunteers following a single oral dose ingested on an empty stomach and with a standardized breakfast (mean \pm S.E. M)

A=500 mg tolbutamide B=250 mg chlorpropamide C=5 mg glibenclamide D=5 mg glipizide

	Time after intake of drug and/or meal (min)								
	0	15	30	45	60	75	90	120	180
A	8 \pm 1	9 \pm 2	10 \pm 2	9 \pm 2	8 \pm 2	9 \pm 2	9 \pm 1	9 \pm 2	7 \pm 1
B	6 \pm 2	5 \pm 2	7 \pm 2	6 \pm 2	7 \pm 2	4 \pm 1	5 \pm 1	4 \pm 1	4 \pm 1
C	11 \pm 3	9 \pm 2	10 \pm 2	11 \pm 2	14 \pm 4	14 \pm 3	11 \pm 2	12 \pm 1	11 \pm 1
D	11 \pm 3	10 \pm 2	14 \pm 3	19 \pm 3	21 \pm 3	20 \pm 4	17 \pm 4	11 \pm 2	11 \pm 1
Meal only	11 \pm 3	24 \pm 6	35 \pm 3	31 \pm 1	30 \pm 4	30 \pm 4	22 \pm 2	17 \pm 3	13 \pm 3
Meal+A	13 \pm 3	31 \pm 4	41 \pm 5	39 \pm 9	25 \pm 5	25 \pm 5	22 \pm 5	18 \pm 3	14 \pm 3
Meal+B	5 \pm 1	27 \pm 5	26 \pm 4	18 \pm 2	16 \pm 4	13 \pm 2	10 \pm 2	9 \pm 2	6 \pm 1
Meal+C	11 \pm 3	33 \pm 7	40 \pm 4	38 \pm 7	40 \pm 3	45 \pm 9	43 \pm 5	32 \pm 5	22 \pm 6
Meal+D	15 \pm 3	35 \pm 8	49 \pm 4	41 \pm 4	41 \pm 5	30 \pm 3	31 \pm 9	30 \pm 6	23 \pm 3

II Blood glucose levels in seven healthy volunteers following a single oral dose ingested both on an empty stomach and after a standard breakfast (mean \pm SEM)

mg tolbutamide B 250 mg chlorpropamide C 5 mg glibenclamide D 5 mg glipizide

Time after intake of drug and/or meal (min)		0	15	30	45	60	75	90	120	140	180
A	fasting	41 \pm 0.1	41 \pm 0.1	39 \pm 0.2	38 \pm 0.1	36 \pm 0.2	36 \pm 0.1	36 \pm 0.2	35 \pm 0.1	34 \pm 0.3	38 \pm 0.1
	meal	44 \pm 0.2	45 \pm 0.2	46 \pm 0.2	44 \pm 0.1	43 \pm 0.1	42 \pm 0.2	42 \pm 0.1	41 \pm 0.1	42 \pm 0.2	45 \pm 0.2
	B	46 \pm 0.2	44 \pm 0.2	42 \pm 0.3	41 \pm 0.2	39 \pm 0.2	36 \pm 0.3	34 \pm 0.2	32 \pm 0.2	33 \pm 0.2	33 \pm 0.2
	C	40 \pm 0.2	41 \pm 0.3	38 \pm 0.3	34 \pm 0.2	28 \pm 0.2	23 \pm 0.2	25 \pm 0.2	28 \pm 0.2	30 \pm 0.2	33 \pm 0.1
B	fasting	43 \pm 0.2	46 \pm 0.2	55 \pm 0.2	50 \pm 0.4	44 \pm 0.3	38 \pm 0.2	39 \pm 0.2	40 \pm 0.2	41 \pm 0.1	42 \pm 0.1
	meal	41 \pm 0.1	44 \pm 0.2	50 \pm 0.3	44 \pm 0.3	34 \pm 0.2	33 \pm 0.2	31 \pm 0.2	36 \pm 0.2	37 \pm 0.2	40 \pm 0.2
	B	46 \pm 0.1	51 \pm 0.2	49 \pm 0.4	44 \pm 0.3	41 \pm 0.3	39 \pm 0.2	40 \pm 0.2	43 \pm 0.2	43 \pm 0.1	50 \pm 0.3
	C	47 \pm 0.2	52 \pm 0.2	59 \pm 0.5	49 \pm 0.6	40 \pm 0.5	33 \pm 0.3	32 \pm 0.3	30 \pm 0.2	32 \pm 0.1	35 \pm 0.1
C	fasting	41 \pm 0.1	49 \pm 0.2	50 \pm 0.3	40 \pm 0.4	27 \pm 0.5	25 \pm 0.3	28 \pm 0.3	27 \pm 0.1	35 \pm 0.1	33 \pm 0.3
	meal	41 \pm 0.1	49 \pm 0.2	50 \pm 0.3	40 \pm 0.4	27 \pm 0.5	25 \pm 0.3	28 \pm 0.3	27 \pm 0.1	35 \pm 0.1	33 \pm 0.3

tolbutamide and glipizide were in the μ mol/l range while those of tolbutamide chlorpropamide were in the μ mol/l range. As tolbutamide and glipizide were at least as active as the two other drugs (vide infra) the support the notion that the intrinsic activity of second-generation sulfonylureas is at least 100 times greater than that of the two first generation drugs.

Fig. 2

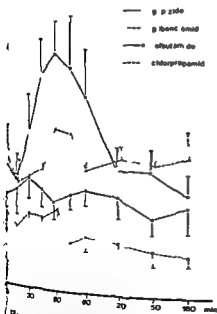


Fig. 2 Plasma insulin concentrations in seven healthy volunteers following intake on an empty stomach of tolbutamide (500 mg), chlorpropamide (250 mg), glibenclamide (5 mg) and glipizide (5 mg). The corresponding figures are given in Table II.

As far as the difference between glipizide and glibenclamide is concerned it was seen that glipizide in the fasting state induced a more pronounced increase in plasma insulin than glibenclamide. Moreover glipizide seemed to promote the greatest blood glucose reduction. Further more hypoglycemic reactions were reported by five subjects after glipizide but by only two after glibenclamide.

These findings could signify that glipizide is a more potent insulin releaser than glibenclamide. However the seemingly stronger effect of glipizide

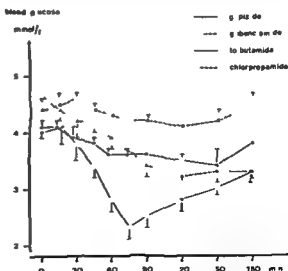


Fig. 3 Blood glucose concentrations in seven healthy volunteers following intake on an empty stomach of tolbutamide (500 mg), chlorpropamide (250 mg), glibenclamide (5 mg) and glipizide (5 mg). The corresponding figures are given in Table III.

may be secondary to biopharmaceutic differences between Min(i)diab® and Daonil® tablets in the same dosage at least 40% more glipizide than glibenclamide reached systemic circulation even though the molecular weights differ by only 10% (446 and 494 respectively). Thus it seems probable that the bioavailability of glipizide from Min(i)diab is greater than that of glibenclamide from currently available Daonil. Accordingly, the question whether glipizide and glibenclamide differ in intrinsic activity remains unanswered by the present study. Another complication in the comparison between the two drugs is that concomitant food intake reduces the absorption rate and hence the effect of glipizide (15) while no such influence has been recorded for glibenclamide (11). A similar tendency was seen in the present study, although the difference did not reach statistical significance.

Another finding that may relate to biopharmaceutic differences concerns the rapidity of effect. Glipizide seemed to be more rapidly absorbed than glibenclamide and the time to reach peak concentration was significantly longer for glibenclamide (3.5 h) than for glipizide (2 h) even though the peak concentration was higher for the latter than for the former. Thus it is likely that glipizide reached its minimum effective concentration more rapidly than glibenclamide. This may explain both the impression that glipizide yielded a more rapid effect and the apparently higher potency of this drug (vide supra). Similarly the slight (tolbutamide) or virtually absent (chlorpropamide) effect of the two first generation sulfonylureas may be due not only to their weaker intrinsic activity but also to slower absorption compared to the second generation drugs.

The present findings confirm that of the four drugs studied chlorpropamide has the by far longest elimination t_1 exceeding 24 h in each subject with a (fasting) mean of 34 h (16). In addition, the estimated t_1 of tolbutamide about 7 h (16) was much longer than those of glipizide and glibenclamide. Furthermore the latter displayed the shortest elimination t_1 of the four drugs with a mean of only 1.8 h (fasting) compared to 4.3 h for glipizide. Assuming that the duration of action of each sulfonylurea is related to its plasma elimination t_1 , chlorpropamide would be the most long acting one and glibenclamide the one with the shortest duration of action. The latter assumption is supported by recent studies suggesting that while the

therapeutic effect of glipizide can be, when this drug is given once daily (4, 7), the effect of glibenclamide is reduced when given once daily (5). On the other hand the concept of glibenclamide as a short acting sulfonylurea is contradicted by much evidence (9) and by the spread clinical experience that glibenclamide provoke long lasting hypoglycemia (7, 4).

The apparent contradiction between the short t_1 and the allegedly long effect of glibenclamide may have several explanations. Firstly it is possible that the sensitivity of the employed methods is not sufficient to allow accurate rate determinations of the true elimination of glibenclamide and glipizide. Indeed for which ^{14}C labelled drugs have been used that both drugs penetrate a deep compartment from which they are only slowly eliminated (11). It can be excluded moreover, that although the metabolite of glibenclamide has a very long elimination half-life in healthy subjects (13) it may accumulate with impaired renal function and hence prolong the effect.

To summarize. Despite the limited possibilities to draw clinically pertinent conclusions from dose studies in healthy volunteers the results support the concept that glipizide and glibenclamide are much more potent sulfonylureas than tolbutamide and chlorpropamide and that this is due to a major difference in intrinsic activity. Glibenclamide appears to be more potent and rapid acting than tolbutamide but this may simply be due to differences in the biopharmaceutics of Min(i)diab and Daonil tablets. Both glibenclamide and glipizide seem to have short elimination half-lives but are probably not informative as regards the duration of action of these drugs.

ACKNOWLEDGEMENTS

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Early Insulin Response in Latent Gestational Diabetes

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RISCT. The intravenous glucose tolerance and stimulated early insulin response (EIR) were 1 in late pregnancy and post partum in a reference (R) group of 9 women and in 18 women with gestational diabetes (LD), defined as a k value ≥ 66 h and a normal fasting blood glucose concentration. During pregnancy, the LD group showed EIR than the R group. However, the response between normal and non detectable. In the pregnant state, the EIR was the same in the two groups. The inability to increase the EIR during pregnancy was most evident in women who even post partum had an abnormal glucose tolerance. In some in the LD group, the EIR during pregnancy was lower than in the non pregnant state. The magnitude of the response in the non pregnant state is decisive of the capacity to increase the secretory response during pregnancy. None of the women in the LD group developed manifest diabetes during pregnancy. At birth to children with normal birth weight, neonatal problems were registered except for 1 child with congenital heart malformation.

Key words: intravenous glucose tolerance test, early insulin response, pregnancy, gestational diabetes.
Acta Med Scand 1980; 208: 309-314.

and delayed glucose stimulated early insulin response (EIR) has been considered a characteristic of all stages of the diabetic syndrome, including prediabetic state, and has been suggested to be prerequisite for the development of manifest diabetes (3). This hypothesis has not been generally accepted, however (1, 14, 16, 23, 24). Pregnancy has a diabetogenic effect on the glucose tolerance due to a decreased peripheral insulin sensitivity (16, 17, 25). An inability to increase the insulin secretion will lead to an impaired glucose tolerance. Even initiate manifest diabetes during pregnancy. Insulin secretion studies in women with gestational diabetes during and after pregnancy will

thus give an opportunity to get further information on the validity of the low insulin response hypothesis.

STUDY POPULATION

Gestational diabetes

Pregnant women with glucosuria during the current pregnancy, a close relative with diabetes or an abnormal obstetrical history in a previous pregnancy were screened for gestational diabetes by means of their fasting blood glucose concentrations. Only women with a relative body mass just before pregnancy of $<120\%$ were included. If two consecutive fasting blood glucose values in late pregnancy were ≥ 5.0 mmol/l, the woman was subjected to an intravenous glucose tolerance test (IVGTT) including EIR measurement. If the fasting blood glucose concentration was <7.2 mmol/l and the elimination of glucose (k value) $<0.66/h$ ($<1/10\%/min$) the woman was considered to have a latent form of gestational diabetes (LD) and was included in the LD group of this study.

This selection procedure gave 16 women. Two women from the reference (R) group (see below) were added to the LD group as they turned out to have subnormal k values. The LD group thus consisted of 18 women aged 20-39 years (median 28). The body mass just before pregnancy was 44-76 kg (median 65) and relative body mass 71-117% (median 101). The body mass gain during pregnancy was 5-30 kg (median 12). The LD group was divided into two equal subgroups on the basis of the blood glucose level 20 min after the glucose injection. Women with the highest blood glucose concentration (>8.4 mmol/l) were subjected to the LD subgroup I and the remaining women to the LD subgroup II.

The women with gestational diabetes were mostly hospitalized from the beginning of the 36th week of pregnancy. They were on a diet with reduced fat and fast carbohydrate content (7.5 MJ = 1800 kcal/day), routinely prescribed to diabetics and exercised regularly. The blood glucose concentration was determined repeatedly. None of the women in the LD group developed hyperglycaemia or ketonuria rendering insulin treatment necessary.

Abbreviations: EIR = early insulin response, IVGTT = intravenous glucose tolerance test, k value = elimination of glucose, LD group = latent gestational diabetes group, R group = reference group.

Table 1 Fasting blood glucose concentration, *k* value, fasting serum insulin concentration and EIR and LD groups in the third trimester and 2 months post partum (mean \pm S D)

	<i>n</i>	Fasting blood glucose (mmol/l)	<i>k</i> value (per h)	Fasting serum insulin (mU/l)	EIR (mU/l)
Pregnant					
R group	9	4.24 \pm 0.40	1.06 \pm 0.41	14.1 \pm 4.7	69.1 \pm 36.5
LD group	18	4.82 \pm 0.85	0.51 \pm 0.10	13.7 \pm 7.3	35.1 \pm 17.2
<i>p</i>		n.s.	n.s.	n.s.	<0.01
Non pregnant					
R group	9	4.43 \pm 0.53	0.96 \pm 0.30	9.5 \pm 3.7	43.4 \pm 16.4
LD group	18	4.86 \pm 0.35	0.79 \pm 0.25	10.2 \pm 3.9*	25.8 \pm 13.6
<i>p</i>		n.s.	n.s.	n.s.	n.s.
Individual difference (<i>p</i>)					
R group		n.s.	n.s.	<0.05	<0.01
LD group		n.s.	<0.01	<0.05	n.s.

n.s. = not significant

* Data missing for one woman each

sary. The foetus was supervised with frequent CTG registrations and determinations of urinary oestriol concentration. The intention was to deliver the women at term.

Reference group

An IVGTT was performed in the third trimester of pregnancy in 11 women without glucosuria during the current pregnancy, a close relative with diabetes or an abnormal obstetrical history in a previous pregnancy. Two of these women had a *k* value of <0.54/h, however, and were included in the LD group. The remaining 9 women, aged 22–38 years (median 31), constitute the R group. Their body mass just before pregnancy was 47–67 kg (median 57) and relative body mass 77–112% (median 88). The body mass gain during pregnancy was 8–22 kg (median 12). The women were delivered at term.

METHODS

A 25 gram IVGTT was performed after an overnight fast during the last trimester of pregnancy as well as two months post partum. The carbohydrate content of the diet on the days preceding the test was normal. Zero time was set when half of the amount of glucose had been injected, i.e. about 14 min after the start. Capillary blood samples for blood glucose determination were collected before the test and at 10, 20, 30, 40, 50 and 60 min. Venous blood samples for insulin analysis were taken before the test and at 2, 4, 6 and 11 min. Serum was stored at -20°C until analysis. Capillary blood glucose was determined by an o-toluidine method (10) and serum insulin by a commercial double antibody radioimmunoassay (Phadebas insulin test[®], Pharmacia, Sweden) (28). The *k* value was obtained from the slope of the total blood glucose concentration from a semilogarithmic plot at the interval 20–60 min using the method of least squares. The *k* value was ex-

pressed per hour (0.6/h = 1.0%/min). EIR was as the mean of the insulin increment above the level at 2, 4 and 6 min. The degree of obesity was expressed as relative body mass, calculated as per cent of ideal body mass according to a height-weight table (19).

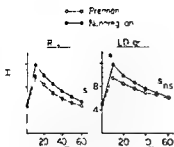
Statistical significance of differences was tested by a non matched paired signed rank test for paired data and by a Kruskal-Wallis *H* test for unpaired observations. A difference was considered significant for values $p < 0.05$ (two-tailed). The Spearman rank correlation coefficient was used to test linear correlation.

RESULTS

Blood glucose. The mean fasting blood glucose concentration was the same in the pregnant and non pregnant state in the LD group and the R group (Table 1). The blood glucose concentration was, however, lower during the first 40–50 min of the IVGTT in the pregnant state than two months post partum in both groups (Fig. 1).

The mean *k* value was unchanged in the pregnant and non pregnant state in the LD group (Table 1). In four out of ten women in the R group with a *k* value of <0.54/h during pregnancy, the *k* value remained as low even post partum and in one woman only a slight increase to 0.62/h was observed. These five women belonged to the LD group. The remaining five women in the LD group had a *k* value post partum in the remaining 11 women in the LD group was 0.90/h.

The mean fasting insulin concentration. The mean fasting insulin concentration was higher during pregnancy than two months post partum in both groups (Table 1). No difference was



Mean blood glucose curves after an i.v. glucose load. $p < 0.01$ (\square) $p < 0.05$ $p < 0.05$ n.s. not significant

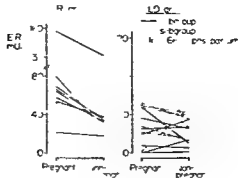


Fig 3 Individual EIR after an i.v. glucose load of 25 g

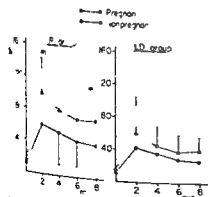
the groups either during pregnancy or post

Insulin response A substantial decrease of β in EIR was observed post partum in women in the R group compared to the insulin response pregnancy (Table I Figs 2 and 3). Except one lean woman with a considerable weight pre pregnancy (75 kg) who had an excellent insulin response the women in the R group showed a moderate insulin response in the pregnant state with peak levels at 2 min of 40-60 mU/l. No correlation was found during pregnancy between the β value and the EIR. Post partum this correlation was 0.77.

Change in EIR from the pregnant to the non pregnant state In the LD group varied (Fig 3) women showed a decrease of the same magnitude as the women in the R group. Others had a similar insulin secretion capacity while still

others had an even higher EIR in the non pregnant state. This is illustrated by one woman in the LD group who had a β value of 0.08/h and an EIR of 7 mU/l during pregnancy. Two months post partum these values had increased to 1.11/h and 48 mU/l respectively. The magnitude of the EIR in the non pregnant state was not decisive for the insulin secretion capacity during pregnancy except for women with extremely low responses and subnormal β values.

The mean insulin concentration during pregnancy in the LD group did not exceed significantly the corresponding value two months post partum until at 8 min owing to a great interindividual variation in insulin secretion capacity (Fig 3). The mean insulin curve during pregnancy was significantly lower in the LD group than in the R group (Fig 4). This difference had disappeared two months post partum. Like in the R group no correlation was found between the β value and EIR in the pregnant state.



Mean S-insulin curves after an i.v. glucose load of 25 g. Statistical symbols as in Fig 1

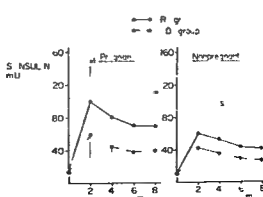


Fig 4 Mean S-insulin curves after an i.v. glucose load of 25 g. Statistical symbols as in Fig 1

Table II *EIR* in LD group women with high (I) and low (II) blood glucose curve

	n	<i>EIR</i> (mU/l)			P
		Mean	Median	Range	
Pregnant					
I	9	22.8	21.0	0-48.7	<0.05
II	9	47.5	48.7	23-105.0	
Non pregnant					
I	8	18.6	19.9	11.3-34.7	<0.05
II	9	32.1	36.0	13.3-47.7	

n.s. = not significant

($r=0.04$ n.s.) A significant correlation ($r=0.39$ $p<0.01$) was however found two months post partum.

As the LD group might be heterogeneous with respect to the degree of impaired glucose tolerance this group was divided according to the height of the blood glucose curve during pregnancy into LD subgroup I with a high and LD subgroup II with a low curve. A significantly lower mean *EIR* was observed in the former subgroup both during pregnancy and two months post partum (Table II). This difference was mainly due to five women who had subnormal *k* values ($<0.66/h$) even post partum (Fig. 3).

Outcome of pregnancy. All women gave birth to living babies including a pair of twins in the LD group. One of the twins died in the neonatal period because of congenital heart malformation. No further perinatal morbidity was noted. The women were delivered at term (\geq gestational week 39) except for two in the LD group in whom labour started spontaneously in week 35-36 (birth weights 2420 and 2500 g). The Apgar score at 1 and 5 min was normal (9-10) in all babies except one with congenital heart malformation.

No significant difference in birth weight was found between children born to women in the LD and in the R group (Table III). Nor was the birth weight increased in children born to women with especially low *k* values, high blood glucose curves or subnormal *k* values in the non pregnant state.

DISCUSSION

The criteria used in this study to screen for gestational diabetes are often considered to indicate a diabetic predisposition (9, 20, 21, 27). Pehrson (21)

found however that women fulfilling these had about the same frequency of subnormal *k* values as the women in his reference group. Women with gestational diabetes are thus also found in traditional risk groups as has also been shown in this study.

The blood glucose curve was lower during IVGTT in the pregnant state in both normal and in women with latent gestational diabetes which might be explained by a larger glucose compartment in pregnancy when the extracellular volume is increased by about 6-7 litres (11). The *k* value in a woman with a low blood glucose might reflect counterregulatory mechanisms rather than a state of truly impaired glucose tolerance. Our LD group might thus be heterogeneous with respect to true latent gestational diabetes. This is further illustrated by the pattern of insulin release (discussed below) as well as the non significant relation between *EIR* and the *k* value during pregnancy. The *k* value should thus not be used as denominator for latent gestational diabetes when the blood glucose curve is clearly elevated. The findings in this study emphasize that the routine for screening of gestational diabetes should be an oral glucose tolerance test as proposed by H. (9).

The normal glucose stimulated insulin release has a biphasic pattern with a fast early and a sustained late phase (2, 7). Both phases as well as the fasting insulin level are normally elevated in certain conditions, for example in obesity (15) and during pregnancy (6, 17, 23, 25) or in an increased peripheral insulin resistance. During pregnancy this decreased sensitivity is thought to be induced by several pregnancy hormones (1). This is in accordance with the increased fasting insulin level as well as the augmented *EIR* in our R group. Patients with manifest diabetes

Table III Birth weights of babies born at gestational week ≥ 39 (1 min post delivery)

	n	Birth weight (g)		
		Mean	Median	Range
R group	9	3 668	3 610	3 070-4 177
LD group	15	3 784	3 800	3 020-4 492
LD subgroup I	11	3 759	3 780	3 060-4 492
LD subgroup II	4	3 814	3 900	3 500-4 111

n.s. = not significant

as well as of the maturity-onset type are characterized by a very low or non-detectable early glucose stimulated insulin secretion (1). This insulin secretion pattern is also found during pregnancy (6). A decreased and delayed glucose stimulated EIR has also been considered characteristic for subclinical stages of the diabetic process including the prediabetic state (3). This leads to the proposal that EIR measurement might be useful in screening programmes for diabetes. EIR has a bimodal and unimodal distribution in the population (11, 16). Very few individuals in the normal population (1–2%) have as low an EIR as patients with manifest diabetes and most of these subjects also have subnormal k values indicating they already have a latent form of diabetes (1). The total risk of developing diabetes during one pregnancy has been calculated to be at least 10–15% (11, 18). It is evident that the magnitude of the decrease during the evolution of the diabetic syndrome from the prediabetic to the manifest stage. Normal EIR has also been found in members of monozygotic twin pairs one of whom had manifest diabetes (4, 5, 26) and in a woman who later developed manifest diabetes (24). The pathogenic effect of pregnancy has been used in studies to obtain further information about the nature of the level of EIR for the development of diabetes.

The most evident finding was the inability to increase the insulin secretion capacity during pregnancy in women who had an impaired glucose tolerance in the pregnant state. This was most clearly seen in the blood glucose curve was high during the pregnancy indicating a more definitive disturbance in glucose metabolism and in women who even during pregnancy had an abnormal glucose tolerance. Of interest was the finding in some women of a decrease in insulin secretion capacity during pregnancy indicating a true β -cell insufficiency. It is not possible to predict the glucose tolerance or the response during pregnancy from the magnitude of the response in the non pregnant state except in women who already then have an abnormal glucose tolerance. It is thus not the magnitude of the response which is important in relation to subclinical forms of diabetes mellitus but the inability to increase or decrease the insulin secretion to situations in which the insulin sensitivity is decreased e.g. during pregnancy and perhaps also in obesity.

For one neonatal death from congenital heart malformation in the LD group. The mean full term birth weight was not increased either in the LD group or in the subgroups with especially low k value, high blood glucose curves or subnormal k values in the non pregnant state. Supervision of pregnancies of women with latent gestational diabetes—including a balanced diet of 75 MJ, exercise, control of blood glucose and urinary oestrogen—as well as delivery at the optimal time—seems to prevent the development of manifest diabetes in the mother and ensures a low perinatal morbidity and mortality and normal birth weight of the child.

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Gangrene Localized to the Lower Limbs in Diabetics

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OBJECT Over a period of eight years 247 un-
 patients with more or less widespread areas
 of cutaneous erythema on the lower legs
 (incipient gangrene) or corresponding
 cutaneous necrosis (manifest gangrene)
 treated at our department. Of these patients
 13% had open diabetes, the others
 studied as non-open diabetics. In 75% of the
 cases these lesions accompanied cardiac de-
 celeration with or without edema, edema of other
 causes—in some cases—arterial insufficiency. The
 developed in most patients a short time
 onset of these precipitating factors. Arterial
 insufficiency alone or together with other precipitating
 factors was seen considerably less often. Edema
 was the main precipitating factor for these lesions
 and decompensation as well as edema of
 due to other causes respond well to treat-
 ment. Treating such patients with open or non-
 diabetes. It should be taken into consideration
 gangrene is a serious condition.

**diabetes mellitus, gangrene, congestive heart
 failure.**

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The incidence of the lower extremities has been re-
 ported to be 156 times more common in diabetic
 non-diabetics in the fifth decade, 85 times
 common in the sixth decade and 53 times
 common in the seventh (2, 9). Gangrene has
 been reported to be the most serious complication in
 a large group of diabetic patients past middle age (1).
 Recently we described a group of elderly pa-
 tients with more or less widespread cutaneous areas
 of erythema with or without necrosis localized to the
 lower limbs (11). Most of them had open dia-
 betes. The oral glucose tolerance test curves of the
 altered in a diabetic direction when com-
 pared with the controls. The latter group of patients thus
 had open diabetes. The cutaneous lesions de-
 veloped most often soon after the onset of cardiac

decompensation with or without edema or after
 edema of other causes. In other words, these dia-
 betic patients had an altered mode of reaction to the
 precipitating factors mentioned when compared to
 non-diabetics. The skin of the lower legs also
 had an altered reaction to experimental local
 traumatization with heat and cold compared to
 non-diabetics (7, 11-16). The altered reaction was
 related to the occurrence of microangiopathy and
 polyneuropathy. The presence of diabetic microan-
 giopathy together with peripheral polyneuropathy
 with impaired capacity to vary the blood circulation
 in the lower limbs (6, 17) was supposed to impede
 the passage of oxygen from the capillary blood to
 tissue cells as well as the passage of metabolites in
 the opposite direction. The presence of edema
 further impairs the passage of these substances.
 Necrosis developed often within the areas of
 cutaneous erythema. We have named cutaneous
 erythema alone incipient gangrene and cutaneous
 erythema with necrosis manifest gangrene.

Gangrene localized to the lower limbs of diabetics
 has been considered to be due to atherosclerosis
 (2, 5, 10). Many authors, however, have asserted
 that this explanation is unsatisfactory and have
 called attention to the fact that these patients have
 microangiopathy and polyneuropathy (3).

For theoretical as well as practical reasons we
 have considered it important to analyse in more
 detail factors of significance for the development of
 different types of gangrene in a large number of
 patients. In the present investigation we have
 examined systematically if there was a close tem-
 poral relationship between the different precipitating
 factors and the development of gangrene.
 Furthermore, the localization of the gangrene to the
 foot alone, foot and lower leg or lower leg alone
 and the factors of importance for the development
 of gangrene in these localizations were studied. Par-
 ticular attention has been paid to the development
 of massive gangrene.

PATIENTS, DEFINITIONS AND METHODS

During 1969-76 we examined 247 unselected patients with gangrene localized to the lower limbs. Cutaneous lesions of the lower limbs consisting of areas of obvious erythema the size of a palm of a hand or larger are designated incipient gangrene. Necrotic skin lesions with the same localization and with involvement of all layers of the skin are called manifest gangrene. In our patients these lesions were almost always surrounded by a zone of erythema. Massive gangrene refers to manifest gangrene of most of the forepart of the foot or the whole heel or larger areas. The classification into incipient and manifest gangrene is based on the appearance of the lesions when they were first observed. Patients with lesions of the medial aspect of the leg were not included in the study because of the common occurrence of varicose lesions in this location. Patients with gangrene caused by arterial embolism were also excluded, as were patients with erysipelas (8/114).

Precipitating factors (Table I) are defined as follows. Cardiac decompensation—a diseased state corresponding to class IV of the functional classification of NYHA (4). Edema—swelling so pronounced that finger impression left a clear impression. Arterial insufficiency—peripheral coldness and absence of pulses in both the dorsalis pedis artery and the posterior tibial artery. Nephrotic syndrome—pronounced proteinuria with hypoproteinemia and edema. In patients with deep venous thrombosis the diagnosis was always verified by phlebography.

Patients with edema of unknown cause are not a homogenous group. Most of them had polyneuropathy and muscular atrophy which ought to impair the venous return due to decreased function of the so called muscle pump (15). This group also includes patients with cardiac decompensation or deep venous thrombosis who did not meet the above diagnostic criteria.

The statement that gangrene developed in close temporal relationship to what are called precipitating factors refers to information from the patient himself, his family, the nurses or the physicians and our own observations. Lack of information of a close temporal relationship does not mean that no such relationship existed.

The significance of differences between means was calculated using Student's *t* test. Fisher's exact probability test was used to test the difference between groups. $P < 0.05$ was chosen as the level of statistical significance.

RESULTS

Patients with non-open diabetes

In the 62 patients without known diabetes oral glucose tolerance tests were performed as described previously (11). The mean blood glucose tolerance test curve area was 15% larger in these patients than in the controls ($p < 0.005$).

Different degrees of gangrene in relation to precipitating factors

Patients with incipient gangrene were compared to patients with manifest gangrene (Table I). The

mean age was about the same in the two groups, namely 68.8 and 67.7 years. Twenty-two (36%) patients were below 50 years of age. Open diabetes was more common in patients with manifest than with incipient gangrene (66%) ($p < 0.05$). Mean duration of open diabetes was significantly longer in patients with manifest than with incipient gangrene (12.1 and 7.9 years respectively) ($p < 0.005$).

Precipitating factors for gangrene were present in 75% of the 247 patients and were more common in patients with incipient (84%) than with manifest gangrene (71%) ($p < 0.02$) and in patients with non-open (82%) than with open diabetes (61%) ($p < 0.001$).

Deep venous thrombosis was more common in patients with incipient (13%) than with manifest gangrene (3%) ($p < 0.005$). Arterial insufficiency was the sole precipitating factor in more patients with manifest (9%) than with incipient gangrene (1%) ($p < 0.02$). Arterial insufficiency and combined with cardiac decompensation was likewise more common in patients with manifest (14%) than with incipient gangrene (4%) ($p < 0.001$).

Edema of all types occurred in 49% of the patients and was more common in patients with incipient (73%) than with manifest gangrene (51%) ($p < 0.001$) (Table I).

Twenty-two patients with massive gangrene were compared with 140 patients with massive or non-massive gangrene (Table I). These patients were in the group of manifest gangrene. Patients with massive gangrene were somewhat older than patients with manifest non-massive gangrene and longer mean duration of diabetes, but the differences were not statistically significant. There were no significant differences in the frequency of the rate above mentioned precipitating factors. Arterial insufficiency included. Nevertheless all precipitating factors together were more common in patients with massive (91%) than with manifest non-massive gangrene (69%) ($p < 0.02$). There were no significant differences when the simultaneous occurrence of precipitating factors was estimated. Cardiac decompensation with and without edema and simultaneous arterial insufficiency was more common in patients with massive (18%) than with manifest non-massive gangrene (14%) ($p < 0.05$). Cardiac decompensation with edema and simultaneous arterial insufficiency (14 and 1% respectively) ($p < 0.005$). All types of edema (14 and 1%

Diabetic patients with incipient gangrene manifest gangrene and massive gangrene localized to the legs and/or feet

	Non open diabetes (n)	Open diabetes (n)				Total	
		Duration of the disease (y) in age group < 60		Duration of the disease (y) in age group ≥ 60		n	%
		<10	≥10	<10	≥10		
Young							
all	79	5	6	36	9	85	
m	17	2	3	16	7	45	
f	12	3	3	20	2	40	
duration of diabetes (y)	69	55	39	73	74	69	
duration of diabetes (y)		3	17	4	16	8	
Young factors							
decompensation							
edema	10	2	1	20 (1)	2	35 (1)	41
no edema	4			3 (1)	1	8 (2)	9
venous syndrome w. th edema			2			2	2
venous thrombosis w. th edema	5	1		3	2	11	13
insufficiency only				1		1	1
of unknown cause	4	1	3	3	3	14	17
total	6	1		6	1	14	17
Elderly							
all	33	11	21	46	41	167	
m	15	6	10	29	22	81	
f	18	5	11	28	19	81	
duration of diabetes (y)	73	49	44	73	68	68	
duration of diabetes (y)		4	23	5	18	17	
Elderly factors							
decompensation							
edema	18 (2)	1		24 (1)	14 (2)	57 (5)	35
no edema	3 (1)	1		9 (1)	6 (2)	19 (4)	12
venous thrombosis w. th edema	4			1		5	3
insufficiency only	4	1	2	3	4	14	9
of unknown cause		1	11	4	4	20	12
total	4	7	8	15	13	47	29
Older							
all	5	1	4	6	6	22	
m	1	1	1	4	3	10	
f	4		3	2	3	12	
duration of diabetes (y)	76	59	48	78	73	70	
duration of diabetes (y)		3	29	5	19	15	
Older factors							
decompensation							
edema	3 (1)			3 (1)	3 (2)	9 (4)	41
no edema					2	2	9
venous thrombosis w. th edema	1			1		2	9
insufficiency only	1	1		1		3	14
of unknown cause			3	1		4	18
total			1		1	2	9

In parentheses indicate number of patients with concurrent arterial insufficiency

are common in patients with massive (68%)
th manifest non massive gangrene (48%)
(Table 1)
In the 247 patients reported trauma as a

fitting shoes as a possible cause of their lesions. We
were not convinced that the traumatization re-
ported was of significant importance for the de-
velopment of gangrene

Table II Data on 108 patients in whom gangrene developed soon after the onset of precipitating factors (n₁) and on 78 patients in whom only a concomitant precipitating factor was registered (n₂)

Precipitating factors	Non-open diabetes				Open diabetes				Total
	Incipient gangrene		Manifest gangrene		Incipient gangrene		Manifest gangrene		
	n ₁	n ₂	n ₁	n ₂	n ₁	n ₂	n ₁	n ₂	
Cardiac decompensation with edema	9	1	8 (2)	10	14 (1)	11	21 (7)	18	4
Cardiac decompensation without edema	2	2 (1)		3 (1)	1	3 (1)	6 (7)	11 (7)	3
Nephrotic syndrome with edema					2				100
Deep venous thrombosis with edema	5		4		6		1		100
Arterial insufficiency only			1	3		1	1	6	79
Edema of unknown cause	2	1			9	2	14	6	4
Total	18	4	13	16	32	17	45	41	44
Precipitating factors with edema	16	2	12	10	31	11	16	24	66
Precipitating factors without edema	2	2	2	5	1	4	9	17	33

Figures in parentheses indicate number of patients with concurrent arterial insufficiency

Temporal relationship between precipitating factors and development of gangrene

Precipitating factors were registered in 186 (75%) of the 247 patients. In 58% of these 186 patients gangrene developed in close temporal relationship to the precipitating factors (Table II).

As regards patients with non open diabetes and cardiac decompensation with edema but without arterial insufficiency a close temporal relationship was found more often among those with incipient (90%) than with manifest gangrene (38%) ($p < 0.02$). As regards patients with non open diabetes and edema of all types together there was also more often a close temporal relationship among those

with incipient (89%) than with manifest gangrene (35%) ($p < 0.02$).

A close temporal relationship between precipitating factors and gangrene was found more often in cases with edema (66%) than without (33%) ($p < 0.001$). As regards patients with edema a close temporal relationship was more often found among those with incipient (70%) than with manifest gangrene (51%) ($p < 0.005$).

Localization of the gangrene

The localization of incipient or manifest gangrene in different parts of the lower limbs is shown in Table III. Manifest gangrene localized to the knee

Table III Localization of incipient and manifest gangrene to the lower legs and/or feet in 247 patients

	Non open diabetes		Open diabetes (n)						Total
			Age groups < 60		Age groups ≥ 60		All ages		
			<10 y	≥10 y	<10 y	≥10 y	n	%	
			n	%	n	%	n	%	
<i>Incipient gangrene</i>									
Foot only	10	34	0	3	14	4	22	39	1
Foot and lower leg	15	52	2	3	20	4	29	57	2
Lower leg foot excluded	4	14	3	0	2	0	5	9	3
Total	29		5	6	36	9	46		16
<i>Manifest gangrene</i>									
Foot only	17	52	4	20	36	30	90	70	17
Foot and lower leg	1	3	1	0	6	5	12	9	11
Lower leg foot excluded	15	45	6	1	14	6	27	21	4
Total	33		11	21	46	41	129		62

cluded was more common in patients with open (45%) than with open diabetes (21%). Manifest gangrene localized to the foot was more common in patients with open than with non-open diabetes ($p < 0.05$). As evident from Table III that the occurrence of gangrene localized to the foot alone in patients with open diabetes was dependent on the type of diabetes. Patients with open diabetes manifest gangrene on the foot alone had a mean duration of diabetes than those with gangrene on the lower leg alone (13.5 and 11.5 respectively) ($p < 0.01$). It is of interest to note that there were no difference in the occurrence of precipitating factors between the 107 patients with manifest gangrene on the foot alone and the 47 patients with gangrene localized to the lower leg alone. The temporal relationship between the precipitating factors and the gangrene was observed more often in the former than the latter patients. The difference was however not statistically significant.

DISCUSSION AND CONCLUSIONS

Patients of this study were collected with the occurrence of characteristic lesions of the limbs. Most of them had open diabetes. Glucose tolerance test curves of the others significantly in a diabetic condition when compared with controls. The lesions described are characteristic of diabetic gangrene. The precipitating factors were demonstrated in 56% of the 247 patients with gangrene. In these 186 patients the lesions developed at the onset of the precipitating factors in 10 percentages are undoubtedly under the explanation above. In the patients with diabetes and edema the latter figure was 90%. In most patients these factors thus the lesions. The precipitating factors are large. The arterial insufficiency in our study are of interest because atherosclerosis has been to be of outstanding importance for the development of diabetic gangrene (1, 2, 5, 10). We consider arterial insufficiency as the sole precipitating factor in 66% of the patients with non-open diabetes and in 45% of those with open diabetes. If patients

with cardiac decompensation with simultaneous arterial insufficiency are added the figure for patients with non-open as well as for those with open diabetes is about 17%. Arterial insufficiency is thus not of outstanding importance for the development of diabetic gangrene. It is to be noted that systemic arterial insufficiency were not more common in patients with open than with non-open diabetes. Arterial insufficiency is however of importance for the degree of gangrene. Arterial insufficiency was the sole precipitating factor in 1% of the patients with incipient gangrene, in 9% of those with manifest gangrene and in 14% of those with manifest gangrene of so-called massive type.

The importance of edema for the development of diabetic gangrene is however sinking. Edema alone or together with other precipitating factors was registered in no less than 58% of the 247 patients and was more common in patients with non-open diabetes and in those with incipient gangrene. A close temporal relationship between development of edema and development of gangrene existed in 66% of these patients.

Both incipient and manifest gangrene in patients with open and non-open diabetes are to be regarded as different degrees of intensity of the same lesions. The degree and duration of diabetes and the different precipitating factors are of importance for the severity and localization of the lesions. It is remarkable that signs of arterial insufficiency were found rather seldom and that gangrene most often developed in connection with edema of different causes.

Our patients are possibly not representative of patients with diabetic gangrene in general. They have all been hospitalized in the Department of Internal Medicine, probably comprising a larger number of patients with gangrene and simultaneous cardiac decompensation than the surgical departments.

Both cardiac decompensation and local edema of the legs due to other causes respond well to treatment. Gangrene being a serious condition this should be taken into consideration when treating patients with open or non-open diabetes. The diagnosis of non-open diabetes is facilitated by these patients who often have characteristic signs and lesions localized to the lower limbs such as polyneuropathy, atrophic skin spots, purpura, non-atrophic pigmentation after previous purpura or redness of the toes (14).

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Elevated Bone Phosphorus/Hydroxyproline Ratio Following Jejunioleal Bypass Surgery

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ACT The degree of bone mineralization, as the bone phosphorus/hydroxyproline (P/Hypro) was studied in 33 patients who had jejunioleal bypass surgery for massive obesity. Low values of bone P/Hypro are expected in the longest postoperative periods ($r_s = 0.65$, $p < 0.001$) with the highest values in patients with vitamin D deficiency, is associated with an elevated degree of bone mineralization or a decreased degree of bone collagen synthesis.

obesity jejunioleal bypass bone phosphorus/hydroxyproline ratio collagen vitamin D deficiency

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Jejunioleal bypass operation causes small bowel resection with intestinal malabsorption of both water and electrolytes (3, 6, 12, 23) and divalent cations (3, 7, 17). The degree of bone composition are complex degrees of osteomalacia with low levels of 25-hydroxycholecalciferol have been demonstrated by histomorphometry in some bypass-operated patients (6, 10, 11, 17). Some investigators have reported increased bone mineral content after bypass surgery (7, 12) whereas others have recently reported normal values (17).

The degree of bone composition can be studied by histomorphometry of large bone biopsies or by measurement of the bone phosphorus/hydroxyproline ratio (P/Hypro) in minute bone biopsies (14). This ratio estimates the degree of bone mineralization which can be taken as an index of osteomalacia. Low values of bone P/Hypro are found in patients with slight vitamin D deficiency

and in patients with severe vitamin D deficiency. We studied the bone disease following jejunioleal bypass operation by determining the bone P/Hypro

PATIENTS AND METHODS

Thirty three patients aged 21-59 years (mean 38) with severe and intractable obesity participated in the study. An average of 5.6 years (range 2-7) before this study they were treated with an end-to-side jejunioleostomy preserving 37.5 cm of the proximal jejunum and 12.5 cm of the terminal ileum. The biochemistry of the patients has been published earlier (12, 14). All patients have received 1200 U of vitamin D₃ daily and calcium supplements of 0.5-1.5 g/day since bypass surgery. The degree of bone mineralization was measured as bone P/Hypro in iliac crest bone biopsies obtained in local anesthesia (24). Duplicate measurements of the biopsies were used to determine the intramethodical variation in the bone P/Hypro. Coefficient of variation was 4.9% (normal 5%) (24). The mean values of the duplicate determinations were used in the study. Bone P/Hypro values for 137 persons without bone disease served as reference values (25). As the normal bone P/Hypro depends on age the ratio was expressed in percent of the corresponding normal mean (25).

Spearman's rank correlation test and median test were used for statistical evaluations.

RESULTS

Table I shows a tendency towards low levels of serum calcium and serum phosphorus. Mean serum alkaline phosphatase is elevated. The mean bone P/Hypro is significantly higher than normal. Only one patient has a subnormal bone P/Hypro (67.1%). Fig. 1 demonstrates that the bone P/Hypro increases significantly with increasing length of the period between operation and measurement ($r_s = 0.65$, $p < 0.001$). No significant correlation was found between bone P/Hypro and either the postoperative weight reduction ($r_s = 0.16$, n.s.) or the frequency of stools ($r = 0.17$, n.s.).

DISCUSSION

The study shows that bone P/Hypro is elevated in patients who have undergone intestinal bypass

Table 1 Serum and bone indices in the 33 patients compared to reference values

	Patients		Reference values		p
	Mean	S D	Mean	S D	
<i>Serum</i>					
Calcium (mmol/l)	2.29	0.20	2.47	0.11	<0.02
Alkaline phosphatase (U/l)	49.2	18.2	31.8	7.2	<0.004
Phosphatase (mmol/l)	1.07	0.5	1.15	0.8	<0.08
<i>Bone</i>					
P/Hypro (% of normal)	105.06	9.6	100.0	7.0	<0.01

surgery. The increase in P/Hypro with increasing length of time since operation indicates progression of a bone disease. The results of serum analysis (Table 1) and a tendency towards low values of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and elevated serum parathyroid hormone reported earlier in some of these patients indicate that they suffer from vitamin D deficiency (12-14).

We determined the bone P/Hypro in order to estimate the average degree of bone mineralization. Phosphorus represents the amounts of mineral and hydroxyproline the amounts of collagen. The ratio has been shown to be an accessible bone index which is low in bone disorders characterized by increased osteoid (2, 27, 29, 30) and it correlates both to indices of importance for bone metabolism (29, 30) and to osteoid determined by histomorphometry (28). Our finding of an elevated bone P/Hypro shows that bypass-operated patients do not develop simple osteomalacia and that the bone disease must be more complex. Absence of osteomalacia in spite of low values of 25-hydroxyvitamin D agrees with the results reported by Davie et al. (8).

P/Hypro is reported to increase with increasing age in normals (25) and in patients with osteoporosis (2), uremia (21, 30) and diabetic eye complications (26). Now we have made the same finding in patients after jejunoileal bypass surgery. The pathogenesis of this hypermineralization is not clear but a defect in vitamin D metabolism has been proposed in the elderly osteoporotic patient (15) and has been proved in uremia (10). The results of the present study may reflect disturbances in

vitamin D metabolism although the bone is not clear.

Theoretically, an increased bone P/Hypro due to the combination of a normal degree of mineralization and elevated phosphorus in bone mineral or a low hydroxyproline in collagen. An elevated P/Hypro may also indicate high amounts of amorphous calcium phosphate with an increased P/Ca ratio in bone (1). Such a condition indicates a high bone turnover (1).

A low hydroxyproline content in collagen could be due to changes in collagen synthesis. A deficiency influences collagen metabolism (19, 20) but changes in the hydroxyproline in collagen have not been reported. Deficiencies of vitamin C and iron, both of which are known to influence proline hydroxylase activity, has been demonstrated in bypass-operated patients. *In vitro* experiments show that the decreased hydroxylase activity in vitamin C deficiency causes pro- α chains deficient in hydroxylation. As such chains cannot be used for collagen synthesis, it is assumed from these results that collagen deficient in hydroxyproline is wasted. Nevertheless, such experiments cannot rule out the possibility that defective collagen synthesis is a minor hydroxyproline deficit can be. Such a defect would explain the high bone turnover in our patients. The increased urinary excretion of hydroxyproline found in gastrointestinal malabsorption (13) may be due to increased bone turnover but it may also indicate a high bone turnover.

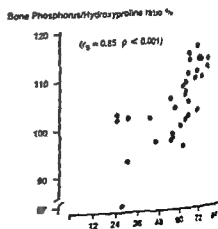


Fig. 1 Postoperative bone P/Hypro in the studied

pro-alpha chains which cannot be used by collagen
 This indicates that patients who have undergone ileal bypass operation suffer from a bone disorder due to changes in collagen metabolism

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Membranous Glomerulopathy in a Patient on Captopril

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ABSTRACT This case report describes a patient with essential hypertension in whom captopril during 18 months resulted in a nephrotic syndrome. A kidney biopsy specimen revealed membranous glomerulopathy stage I. After withdrawal of the drug, urinary protein loss disappeared within ten days. However, a second biopsy three months later showed granular deposition of IgA, IgG, IgM, and C3 in the glomerular basement membrane and subepithelial electron dense deposits on immunoelectron microscopy. Glomerular filtration rate remained normal during the observation period.

Key words: captopril, converting enzyme inhibition, chronic glomerulopathy, nephrotic syndrome.

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The converting enzyme inhibition by captopril (1 (D,L)-2-methyl-1-oxypropyl-L-proline) appears to be very effective in the treatment of hypertension (3, 15) in some of the patients not only after combination with sodium dechlorate (3, 15). Reduction in blood pressure (BP) by captopril therapy results in a gradual reversal of the blockade of angiotensin II formation (1, 15). The drug also inhibits extrapulmonary generation of angiotensin II (13) remains to be elucidated. The same holds for the contribution of captopril to the diminished bradykinin hydrolysis to angiotensin II (1, 18).

Known side effects of captopril include fever (3, 7, 13, 15), angioneurotic oedema (15), aphthous ulcers of the mouth (17) and eosinophilia (7). Most of these side-effects are reversible (3, 7, 15, 19). Nephrotic syndrome and renal insufficiency have also been reported occasionally in patients on captopril (10).

This case report describes in detail the first patient with essential hypertension in whom nephrotic syndrome developed while on captopril. Secondly, an account of the effects of withdrawal of captopril treatment on urinary protein loss and morphological changes in the kidney of this patient will be given.

CASE REPORT

The patient was a 48-year-old woman in whom hypertension (155/100 mmHg) was found in 1965. Her medical history included an appendectomy and a hysterectomy. Two pregnancies had been uneventful. Her mother had also suffered from hypertension.

In 1977 the patient was referred to our Outpatient Clinic because her BP was 160/140 mmHg supine and 170/150 mmHg upright despite treatment with sodium restriction, chlorthalidone and α -methyl dopa. Chest X-ray and ECG showed no abnormalities. Fundoscopy revealed hypertensive changes of K-W grade II. Rapid sequential urography, renography and renal angiography were normal. Without therapy serum sodium was 139 mmol/l, serum potassium 4.3 mmol/l and serum creatinine 77 μ mol/l. Vanillylmandelic acid excretion was normal. The urine contained neither protein nor glucose and the urinary sediment was normal. A diagnosis of essential hypertension was made and she was treated with sodium restriction, hydrochlorothiazide, potassium supplement and clonidine.

In Oct. 1978 the antihypertensive therapy was discontinued because her BP control was unsatisfactory (170/170 mmHg supine). The BP rose to 170/140 mmHg. At that time serum sodium was 139 mmol/l, serum potassium 4.2

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Abbreviations: ANA, anti-nuclear antibodies; ERPF, effective renal plasma flow; FF, filtration fraction; GBM, glomerular basement membrane; GFR, glomerular filtration rate; PAC, plasma aldosterone concentration; PRA, plasma renin activity; MGP, membranous glomerulopathy; BP, blood pressure.

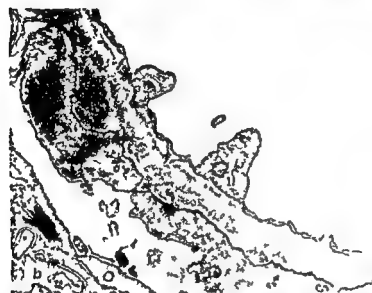
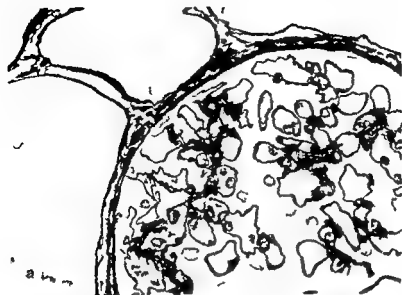


Fig 1 (a) Light microscopy (b) electron microscopy and (c) immunofluorescence microscopy ($\times 144$) of the resection biopsy in May 1979

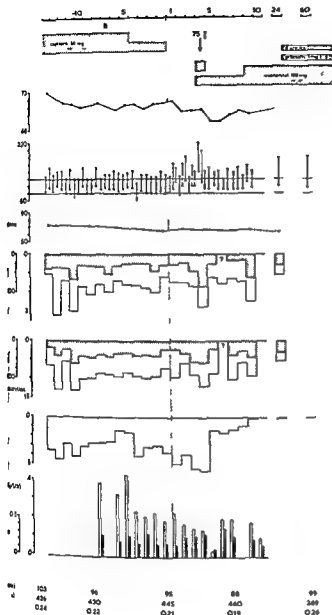


Fig 2 Effect of withdrawal of captopril on BP, urinary protein loss, PRA (□), PAC (◻) and renal function. 75 γ C=75 gamma clonidine. H=50 mg hydrochlorothiazide.

and serum creatinine 71 μ mol/l. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were 103 and 430 ml/min respectively. Filtration fraction was 0.24. The urine contained no protein and the sediment was normal. Antinuclear antibodies (ANA) were not found. Supine plasma renin activity (PRA) amounted to 0.8 nmol A_p /l/h and plasma aldosterone concentration (PAC) to 0.47 nmol/l (12–14). An arterial blood pressure (BP) control (130/80 mmHg) was obtained with 50 mg captopril daily and the patient remained normotensive between monthly visits to the Outpatient Clinic. GFR increased to 426 ml/min.

In March 1979, slightly positive (titers 1/10) homogeneous pattern) were disclosed. Antibodies

against native DNA were absent. In April 1979, the patient noticed oedema of the feet. Proteinuria was present (5 g/24 h). On admission in May 1979, her BP was normal (120/80 mmHg) as was the urinary sediment. Hypoalbuminaemia (2.6 g/100 ml) and hypercholesterolaemia (10.4 mmol/l) existed. Serum creatinine was 74 μ mol/l, GFR 96 ml/min, ERPF 430 ml/min and FF 0.22. ANA were positive (titer 1/10) but antibodies against native DNA negative. Complement (C_{3a}) amounted to 124% of standard serum. There was no cryoglobulinaemia. A renal biopsy was performed and no proliferative or exudative changes were found on light microscopy (Fig 1a). Electron-dense deposits along the subepithelial side of the glomerular basement membrane (GBM) were revealed on electron micro-

scopy (Fig 1b) Immunofluorescent studies showed IgA IgG IgM and C₃ distributed in a granular pattern in the GBM (Fig 1c)

Captopril was discontinued and BP rose thereafter to preexisting values (Fig 2) PRA decreased and PAC increased Hypertension was now treated with hydrochlorothiazide triamterene metoprolol and prazosin A satisfying BP control was achieved Interestingly proteinuria disappeared completely within 10 days after withdrawal of captopril (Fig 2) A second biopsy at the end of Aug 1979 showed unchanged immunofluorescence and unchanged subepithelial electron-dense deposits Light microscopy was normal again At that time there was no proteinuria Urinary sediment was normal ANA had disappeared GFR amounted to 99 ml/min ERPF to 369 ml/min and FF to 0.26

DISCUSSION

The renal biopsy findings in our patient are consistent with the diagnosis of membranous glomerulopathy (MGP) stage I (5) We have made similar findings in another patient who developed a captopril induced serum sickness like syndrome (10) Proteinuria during captopril treatment has also been noticed by other investigators who found an early stage of MGP in the renal biopsy specimens as well (dr J Knill personal communication) Thus the occurrence of MGP in patients on captopril is unlikely to be fortuitous and patients taking this drug should be carefully monitored However the frequency of proteinuria during captopril treatment is hardly 1% and can be transient despite continuing administration of the drug (Squibb & Sons Inc) Moreover renal function (i.e. GFR) seems not to be compromised (9) The way in which captopril induces MGP in some patients is unknown The similarity of this immune complex glomerulopathy to MGP induced by D penicillamine is however striking

It was further remarkable that the proteinuria in our patient disappeared completely within 10 days after withholding the drug In other drug induced immune complex glomerulopathies for instance D penicillamine induced proteinuria disappears after a much longer time lag and more in accordance with the disappearance of the morphological changes Therefore we wonder if captopril by decreasing the generation of angiotension II and by inhibiting the degradation of bradykinin might facilitate glomerular protein loss when immune complex deposition has taken place In this respect it is noteworthy that the protease inhibitor trasyolol reduces aminonu-

cleoside induced proteinuria in the rat (11) and inhibits generation of kallikrein and thereby the generation of bradykinin It is even possible that the pharmacological action of captopril may have played a permissive role in the deposition and formation of immune complexes The way in which these immune complexes remains however is not elucidated

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Pseudotumor Cerebri in Pseudohypoparathyroidism

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ACT A case of hypocalcemia with elevated TH but normal renal response of cAMP and to PTH infusion is reported. The patient had papilledema since many years probably due to pseudotumor cerebri.

di pseudotumor cerebri pseudohypoparathyroidism

Scand J Med 1980

Pseudohypoparathyroidism (PHP) is the first disorder in which a defective response to a hormone in a normally functional organ has been recognized (1). It is characterized by an inadequate phosphaturic response to exogenous parathormone. The patients generally have typical dysmorphic features and laboratory signs of hypoparathyroidism resistant to PTH. Cyclic adenosine monophosphate (cAMP) in urine has also been found to be resistant to PTH. This has been used as a test for PHP. A few PHP patients with a normal response of cAMP to PTH but with a normal phosphaturic response have however been reported. This type of PHP has been called (2) variant PHP with hypocalcemia and elevated PTH but normal renal response (phosphate and cAMP). This type of PHP has recently been reported (5). The patient had reduced levels of 1,25-dihydroxycholecalciferol. Therapy with 1,25-dihydroxycholecalciferol restored the calcemic response to PTH to normal. This could be explained by skeletal but not by renal resistance usually present in ordinary PHP.

CASE REPORT

The patient, a 45-year-old man, had tetanic symptoms probably since childhood and during childhood and adolescence. He was treated in 1936 with the diagnosis of tetany. At that time his serum calcium was 6.3 mg/100 ml. He was given vitamin D for a short time and has since then had no tetany or other neurologic symptoms.

In 1952 the patient was admitted because of headache. He had no neurologic symptoms or signs. A papilledema with a protrusion of 3-4 diopters was found. Because of this papilledema he was then on several occasions investigated with carotid angiographies as well as encephalography and ventriculography. No cerebral tumor could be found but the papilledema persisted. Serum calcium was not checked at that time. In recent years the patient has been unable to work because of lumbar pains and obesity.

Hypocalcemia was noted once again in Feb 1976. The sole symptoms possibly related to this hypocalcemia were paresthesias in the ulnar fingers of both hands. Physical examination revealed an obese man with a weight of 121 kg and height of 178 cm. Normal hands, no Chvostek's signs, slight hypertension, no cataracts. X-ray of the skull revealed no calcifications of the basal ganglia. X-rays of the lumbar columna and hands were normal apart from spondylarthrosis. Except for hypocalcemia 1.8-2.0 mmol/l, the routine laboratory investigations showed normal findings. Serum phosphate, serum magnesium and hematology were normal. Treatment with calcium gluconate 2 g/day and dihydrotachysterol 0.4 g twice daily was initiated. The therapy continued for six months but was withheld one week before the first investigation. The same treatment was reinstituted and continued for another two months and was withheld another ten weeks before the second investigation.

METHODS

Food was withheld for 12 hours but water intake ad lib was permitted before the study. On the day of the study a Foley catheter was inserted in the bladder. The patient drank 250 ml water hourly from 6:00 a.m. until noon. Urine was collected at 30-min intervals from 8:00 a.m. till noon. PTH 300 USP in 50 ml of 0.9% sodium chloride and 0.5% human serum albumin were infused at 9:00 a.m. The same procedure was carried out in a volunteer without signs of renal or parathyroid disease. Blood samples for calcium and phosphorus before and after PTH administration were unfortunately drawn only in the last study. Urine portions were immediately analyzed for calcium and inorganic phosphorus. PTH was estimated according to Almqvist et al. (2). The method of Steiner et al. (6) was used for determination of cAMP.

Abbreviations: PHP=pseudohypoparathyroidism, PTH=parathormone, cAMP=cyclic adenosine monophosphate.



Fig 1 Liver biopsy 5 months of carbamazepine treatment showing changes with hyperplastic epithelial cells and nuclei of giant cells.

vacuolization but no granulomas. Mitochondrial antibody examination (not performed during the first admission) was positive 1/100. Five months later this test was negative. Antibodies against nuclei and smooth muscle were negative. Bacteriological and serological tests for tuberculosis, toxoplasmosis, mononucleosis, viral hepatitis, cytomegalovirus, mycoplasma, parainfluenza, influenza, adenovirus, ornithosis were negative.

DISCUSSION

This case report concerns a patient with reversible granulomatous hepatic reaction. There are good reasons to associate the disease with coincidental carbamazepine treatment. The illness started 3

Table 1 Sequential changes in pertinent biochemical findings

	Normal values	Jan 15	Jan 25	Feb 2 ^a	Feb 22
ESR (1 mm/1 h)	2-15	22		39	7
Leukocytes ($\times 10^9/l$)	4-9	13.1	9.4	9.5	6.0
Eosinophils ($\times 10^9/l$)	40-440	650		523	120
Total bilirubin ($\mu mol/l$)	4-21	17.1	3.8	5.3	9.0
ALP ($\mu kat/l$)	0.8-4.8	8.9	5.0	1.7	1.6
ASAT ($\mu kat/l$)	<0.6	5.0	0.4	0.7	0.3
ALAT ($\mu kat/l$)	<0.6	4.5	0.7	0.8	0.3
LDH ($\mu kat/l$)	3.8-6.7	11.1			6.1

^a Carbamazepine given Dec 18 1978-Jan 16 1979 (30 days). ^b Carbamazepine given Jan 29 1979-Feb 1 1979 (4 days).

weeks after initiation of the drug. No ed was given during this time. The liver disease disappeared in less than one week after cessation of the drug. During a 4-day provocative carbamazepine was reinstituted and the symptoms with pain, fever and elevation of ALAT reappeared. Reversible elevation of enzymes is a known side-effect of the drug. Other explanation of this reversible granulomatous hepatic disease could be found neither logically nor serologically.

One question to be answered is: Can granulomas be formed during a 4 week period? The granulomas induced by phenylbutazone or oxyphenylbutazone have shown a formation period (up to 24 days) and lack of regression with dosage (1.8-70.0 g) (3). In an experimental model I have observed the formation of giant cells in 13 days (4). In this case it seems quite possible that one exposure to carbamazepine treatment may result in the formation of granulomas.

The reversible appearance of antibodies is interesting from an immunological point of view. Do these antibodies play a pathogenetic role in this disease or are they as a result of a toxic unspecific hepatic reaction? It is not possible to comment on this question at the present time.

The prognosis in this type of drug-induced liver disease seems to be favorable if administration of the drug is discontinued in due time. The liver

se showed complete disappearance of the granulomas and reports of serious liver necrosis of this type are rare. This report and others (5) emphasize the necessity of tests for hepatic dysfunction in patients receiving carbamazepine.

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ANNOUNCEMENTS

The French Association of Haemophiliacs International Prize of F.F. 15 000 will be awarded for the fourth time in 1981. The regulations in French or English will be forwarded on request by the Selection Committee Association Française des Hémophiles C.N.T.S. 6 rue Alexandre Cabanel 75739-Paris Cedex 15 France. The work submitted for the Prize must reach the Secretariat of the Selection Committee by March 15, 1981 at the latest.

The British Journal of Pain will commence publication early in 1981.

As pain is a ubiquitous and important symptom in many diseases it is hoped that this new Journal will arouse interest in a wide range of medical disciplines. The inten-

tion is to provide a forum for original work & articles related to the cause and treatment of pain. The primary aim of the new Journal is to publish of a practical clinical nature although basic (animal or laboratory) and philosophical papers may be refused.

The Journal will have an independent Editorial Board not directly connected with any Society and will appear quarterly.

The Editorial Committee will be pleased to receive papers for publication. Instructions for authors and subscription forms are available from the Editor and Publisher British Journal of Pain, John Sherman & Co., 78 Park Road, Altrincham W.A.14 6QQ, England.

CONTINUED DEBATE

Dietary Fatty Acids and Ischaemic Heart Disease

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Importance of prostacyclins in the prevention of thrombosis has led to interest in the role which fatty acids which are precursors of prostacyclin play in the prevention of ischaemic heart disease. An increase in certain fatty acids in the diet advantageously influence platelet function.

Formation of prostacyclins

Platelet aggregation represents an early event in the chain of events which leads to the formation of a thrombus. Two groups of clinical compounds which affect platelet aggregation are the prostacyclins (PGI₁) and thromboxanes (TxA₂). Prostacyclins are formed by the endothelial linings of many blood vessels and are the most potent inhibitors of platelet aggregation yet discovered. In addition prostacyclins are vasodilators. Thromboxanes on the other hand are produced by platelets and promote their aggregation and are also vasoconstrictors. A balance between the formation and activities of these two substances, prostacyclins and thromboxanes, is important for the maintenance of the cardiovascular system (1). An imbalance in the favour of thromboxanes will result in an increased tendency to thrombosis and an imbalance in the favour of prostacyclins a tendency to bleeding. It is of relevance that prostacyclin production has been shown to be reduced in human atherosclerotic plaques (1).

Formation of prostacyclins

Thromboxanes and prostacyclins are produced from the same polyunsaturated fatty acids but by different enzymes. There are three different types of compounds in the 1, 2 and 3 series each arising from a different polyunsaturated fatty acid. Arachidonic acid (C20 4w6) and eicosapentaenoic acid (C20 5w3) are precursors of two different types of prostacyclins and thromboxanes (Fig. 1).

The former arachidonic acid originating from the w6 fatty acids gives rise to a prostacyclin (PGI₁) and a thromboxane (TxA₂). The latter eicosapentaenoic acid originating from the w3 fatty acids produces a prostacyclin (PGI₃) and a corresponding thromboxane (TxA₃). Both prostacyclins (PGI₁ and PGI₃) are antiplatelet aggregating agents. However TxA₃ derived from eicosapentaenoic acid is less active as a promoter of platelet aggregation than TxA₂. If therefore the pathway which predominates in any individual preferred the formation of PGI₃ and TxA₃ the haemostatic balance might be shifted away from the thrombotic side of the system thus affording some protection against thrombosis.

The essential difference between the two series PGI₁, TxA₂ and PGI₃, TxA₃ resides in the nature of the third bond counting from the terminal methyl group of the fatty acid molecule in the w3 series this bond is double whereas it is single in the w6 series. The conversion of fatty acids of the w6 series into fatty acids of the w3 series does not occur in man.

Clinical studies of the role of prostacyclin precursors

Although diets containing different fatty acids may affect cholesterol levels and atherosclerosis in the same way they may have different effects on thrombogenesis. Epidemiological studies of the incidence of ischaemic heart disease have led to some appreciation of the importance of diet in the aetiology of ischaemic heart disease.

Deaths from cardiovascular disease are rare among Eskimos and this has been attributed to an increased bleeding tendency which they possess. Eskimos consume large quantities of w3 polyunsaturated fatty acids, precursors of PGI₃ and platelet lipid analysis has demonstrated an increased proportion of these polyunsaturated fatty

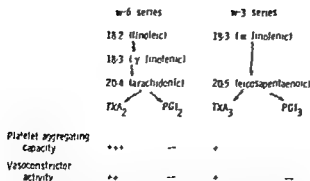


Fig 1 Metabolism of some polyunsaturated fatty acids. Number of positive or negative signs indicate the potency of the compound in promoting or inhibiting the particular physiological action

acids in the platelets (3). In particular platelet eicosapentaenoic acid levels in Eskimos are markedly elevated when compared to the levels found in Danes in whom ischaemic heart disease is relatively common (Table 1). Thus the low incidence of myocardial infarction in Eskimos may result from the increased synthesis of PGI₂ from eicosapentaenoic acid resulting in an alteration of the balance between aggregation and inhibition of aggregation of platelets towards the latter.

Alteration in the diet to achieve a similar fatty acid pattern as Eskimos may therefore prevent the development of coronary thrombosis. A study of the administration of eicosapentaenoic acid to volunteers in their diet in the form of 650 g mackerel per day has shown that after only one week on this diet considerable changes in the fatty acid concentration in the plasma and platelets occurred (16). A

Table 1 Percentage concentration of eicosapentaenoic acid

Group	C20:5w3 (%)	Source
Platelets		
Eskimos	8	Dyerberg & Bang (3)
Danes	11.5	
Controls	1	Siess et al (16)
After mackerel diet for 6/7	5.1	
Erythrocytes		
Controls	6	Lea et al (18)
Patients with ischaemic heart disease	3.9	

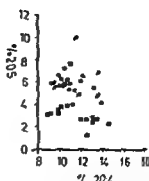


Fig 2 Fatty acids of the lipids of human erythrocytes. Plot of % C20:5 versus C20:4. ● = Patients from myocardial infarction. ○ = controls. The apparent relationship between % C20:4 and % C20:5 in heart patients is 1.9 in controls 11.06 ± 0.29 (mean ± S.E.M.). In the graphic analysis the peak containing C20:4 may be small. Subsequent studies have separated these two peaks shown that the latter is small.

marked decrease in arachidonic acid and increase in eicosapentaenoic acid resulted associated reduction in platelet aggregation, TXA₂ synthesis (Table 1).

Further support for the importance of eicosapentaenoic acid in thrombosis has come from a study using the erythrocyte membrane as probe to compare the fatty acid composition of 20 patients aged below 65 years with myocardial infarction and a group of matched healthy controls (18). There was no significant difference between the level of arachidonic acid in the patients and controls but there was however a highly significant difference in the level of eicosapentaenoic acid. Markedly depressed values were found in those patients who had had myocardial infarction (Table 1) and Fig 2).

What of the relationship between previous studies and newer theories of the role of prostaglandins and thromboxanes in the aetiology of coronary thrombosis? Low density lipoproteins correlate positively with ischaemic heart disease (6) have been found to inhibit in vitro platelet aggregation by human endothelial cells. Moreover high density lipoproteins correlate negatively with ischaemic heart disease and are increased by fish which is rich in w-3 fatty acid precursors of PGI₂ (10). The latter two findings show benefit from diets rich in polyunsaturated fatty acids may be due to an increased production of polyunsaturated fatty acids.

II Ratio of w6 :w3 fatty acids and percent eicosapentaenoic acid in various foods

	Ratio w6 :w3	C20 :w3 (%)
	0.22	17.8
	0.29	14.3
	0.13	6.0
	0.42	7.1
m	0.13	13.5
	0.36	9.6
	0.06	15.8
	0.28	11.9
	0.38	14.0
	0.34	13.2
	0.34	10.8
	7.3	Trace
	19.0	Trace
	1.0	Trace
	8.2	Trace
	0.9	-
	100	-
	72	-
	12	-
(zero erucic)	2.0	-
	147	-
(Northern)	220	-
	7.5	-

Dietary recommendations

In the past forty years the incidence of ischaemic heart disease has considerably increased. In the same period the British diet has changed markedly. In particular the w6 :w3 ratio of the diet has gradually increased mainly because of the increased consumption of linoleic acid rich plant oils in the form of margarine and cooking oils but to a lesser extent due to a decreased consumption of animal fats. It has been calculated that over the last forty years the w6 :w3 ratio has increased from 6 :1 to 20 :1. A return to the diet of yesteryear might be beneficial in the prevention of thrombosis. This can be achieved in two ways. Firstly an increase in the intake of fatty acids of the w3 series together with a decrease in the fatty acids of the w6 series would result in an increased synthesis of PGI₂ and a decrease in TxA₂. Table II shows those foods which are a low ratio of w6 :w3 fatty acids in particular which might be beneficial to achieve this. However controversy still surrounds the

question as to whether elongation and desaturation of α -linolenic acid (C18 :w3) does indeed occur in man (4, 15). If it does occur then enrichment of the diet with α -linolenic acid (e.g. olive oil, soybean oil) would provide the necessary fatty acid for PGI₂ synthesis.

The other alternative is to increase in the diet those foods rich in eicosapentaenoic acid which as can be seen from Table II are mainly fish. Although it has been claimed that diets rich in polyunsaturated fats may be associated with cholelithiasis there is no evidence that they are carcinogenic (5).

Prostacyclins in other diseases

Increased prostacyclin synthesis by dietary modification may be beneficial not only in the prevention of coronary thrombosis but also in other medical conditions. Prostacyclin production by blood vessels from patients with diabetes is depressed (7, 17, 18). It has been suggested that vascular complications in such patients may be due to deficient prostacyclin production and enhanced platelet aggregation and adhesiveness. Low concentrations of 6-Oxo-PGF_{1 α} , a metabolite of prostacyclin, have been found in diabetics (2).

In the haemolytic uraemic syndrome characterized by widespread microthrombosis predominantly in the kidneys, a deficiency of prostacyclin production by the vascular endothelium has been found (14, 20).

Of recent interest has been the role of prostacyclin in pregnancy and the possibility that a relative deficiency of prostacyclin might be a factor in the pathogenesis of pre-eclampsia (9). A reduction of foetal vascular prostacyclin activity has been shown in those pregnancies complicated by pre-eclampsia (13).

Conclusion

Before a change in diet is recommended for patients with ischaemic heart disease or for populations at risk of developing ischaemic heart disease more evidence is required. There are however some indications that diets rich in eicosapentaenoic acid may be beneficial by virtue of the role of this fatty acid in the synthesis of PGI₂. Dietary control of the function of the platelet and prostacyclin production by the vascular endothelium may be a useful addition in the prevention

there may be other diseases in which endogenous production of prostacyclin might be beneficially increased

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LETTER TO THE EDITOR

The Lipid Hypothesis

(With apologies to W. Shakespeare)

Friends, clinicians, colleagues, lend me your eyes
 I come to bury the Lipid Hypothesis, not to praise it
 The errors that men publish live after them
 The good is oft interred with their bones
 So let it be with lipids. The noble Werko (8)
 Hath told you the Lipid Hypothesis was false
 If it were so, it was a grievous fault
 And grievously hath lipids answered it
 Here, under leave of Werko and the rest
 (For Werko is an honourable man
 So are McMichael (6) and Mann (5), honourable men)
 Come I to speak in the Lipid Hypothesis funeral
 It was Malmros' (4) belief, logical and acceptable to me
 But They say it was specious
 And They are honourable men
 Lipids brought forth support from industry
 Whose funds did some research coffers fill
 Did this make lipidologists ambitious?
 When hypercholesterolaemics have died young, we have wept
 Ambition should be made of sterner stuff
 Yet They say we were ambitious
 And They are honourable men
 You all did see that the American Heart Association
 Thrice issued reports on diet, and a fourth (1)
 Which Oster (7) did refute. Was this ambition?
 Yet They say it was ambitious
 And sure, They are honourable men
 I speak not to disprove what They wrote
 But here I am to write what I do know
 Many of you did hold it once, not without cause
 What cause withholds you then to mourn for it?
 O judgement, thou art hard on epidemiology (3)
 While nutritionists, seemingly, have lost their reason (2)' — Bear with me
 My heart is in the coffin there with the Lipid Hypothesis
 And atheroma must regress before it come back to life

Gilbert Thompson, MRC Lipid Metabolism Unit
 Hammersmith Hospital, London, England

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BOOK REVIEWS

Vitamin D—Metabolism and Function By H F DeLuca DM 29 - US \$16.00 Springer Verlag Berlin Heidelberg and New York 1979

The last decade has brought a wealth of information on vitamin D and its role as a prohormone is now firmly established. One of the foremost investigators in this field Dr Hector F DeLuca has written a comprehensive monograph on the metabolism and function of vitamin D as it is currently understood.

In his introduction the author states that the monograph could be looked upon as a progress report of a study of the metabolism and function of vitamin D. True to this statement DeLuca in his authoritative overview repeatedly reveals that although a lot is known there is still a lot more to be known concerning vitamin D. This is especially true regarding the regulation of the metabolism of vitamin D and the role of the various metabolites in the complex homeostasis of calcium and phosphate.

After a short well written historical introduction the D vitamins and their precursors are presented before the author thoroughly discusses the metabolism of vitamin D and the regulation of this metabolism with special emphasis on the kidney as an endocrine organ. The reader is then given insight into the function of vitamin D in the intestine, bone and kidney. Analogs of vitamin D metabolites and the use of vitamin D compounds in clinical medicine are discussed.

The text is easy to read and the author provides a broad and comprehensive perspective of this rapidly expanding field and gives access to a large literature.

Although much of this book is too specialized for most practitioners of medicine it should be of considerable interest and value to those with special interests in the field.

Lars Teyler and John Fredrik Dvinge

Renal calculus By L N Pyrah DM 89-1 Springer Verlag Berlin Heidelberg and New York 1979

Professor Leslie N Pyrah distinguished clinical researcher has written a monograph that covers field of renal calculous disease. The author is as a urologist and researcher in a department concerned with the investigation of calculous diseases. This monograph is written from a broad point of view.

The volume contains 17 chapters dealing with epidemiological, pathological, biochemical, medical aspects of the often encountered renal stones in the urinary tract. It is written from a clinical standpoint and reflects the author's extensive experience.

The illustrations are good, the pages are well laid out and the print is easy to read. References are plentiful and conveniently located at the end of each chapter. A detailed list of contents, the use of subheadings, an excellent index make it easy for the reader to find what he is looking for.

According to the author the increase of renal calculi available in recent years has necessitated an updated presentation of the subject. The reader however will be impressed by the wealth of information as well as clinical contained in this book. It is strongly recommended to internists, renal urologists and everyone else interested in renal medicine.

Lars Teyler and John Fredrik Dvinge

Sinus Node Dysfunction in 128 Patients

A Retrospective Study with Follow up

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From Department of Medicine B, Odense University Hospital, Odense, Denmark

ABSTRACT A retrospective study covering all admissions during a 6-year period revealed 128 patients with sinus node dysfunction (SND). The patients were divided according to the ECG criteria chosen into three groups: group I 37 with sinus bradycardia, group II 37 with sinoatrial block/sinus arrest, group III 58 with brady-tachy syndrome. Additional heart disease, mainly ischaemic, was found in 56%. The frequency and severity of symptoms increased from group I to group III. Pacemaker treatment was given in 17% of the cases, while medical treatment alone was successful in 17%. A follow up including 104 patients was carried out after a mean observation time of approximately three years. Sixteen patients died. The cause of death may have been SND in only one case. Five patients died of apoplexy or complications to such. In total, nine deaths or proven systemic embolic events were all occurring in patients with brady-tachy syndrome. A progression of the ECG abnormality from a lower to a higher group took place in nine patients during the observation period. It is concluded that SND is a condition with a broad clinical spectrum and a stationary or slowly progressive course. In general, it carries a good prognosis. A substantial number of deaths or disabilities in patients with brady-tachy syndrome may be ascribed to systemic embolism. Long term anticoagulant therapy is needed in this subgroup of patients with SND.

Key words: sinus node dysfunction, syncopal attacks, pacemaker treatment, systemic embolism.

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Increasing number of publications on sinus node dysfunction (SND) have appeared during the last few years. The main objects of interest being electrocardiological phenomena (2, 3, 8) and the possibilities of treatment (12, 15, 17). The syndrome is

accompanied by symptoms varying considerably in severity. There are only a few reports regarding the clinical spectrum (7, 15, 16) and these include smaller numbers of patients.

A study of 128 patients is presented in an attempt at defining the difference between patient groups obtained from ECG criteria with regard to the occurrence of symptoms, need of treatment, risk of progression and prognosis.

PATIENTS AND METHODS

The study comprises 128 patients (74 men and 54 women) selected retrospectively by means of a survey of the case reports of all admissions to Medical Department B, Odense University Hospital, from April 1, 1972 to Dec 31, 1977. Telemetry, but not Holter technique, was employed in detection of arrhythmia and in evaluation of antiarrhythmic therapy. The patients were selected on the basis of ECG findings in accordance with the criteria mentioned below. Excluded from the study were patients with acute myocardial infarction or SND caused by antiarrhythmic treatment.

The patient series was divided into three groups on the basis of the following ECG criteria: *Group I* Persistent and in other respects unexplainable sinus bradycardia with a heart rate of less than 50/min at rest. *Group II* Sinus rhythm with episodes of sinoatrial block or sinus arrest. *Group III* Patients with characteristics as above and at least one episode of supraventricular tachyarrhythmia (brady-tachy syndrome).

A follow up examination was carried out by one of us (E.S.) in Sept.-Oct. 1978 after an average observation time of about 3 years. The follow up included an interview regarding symptoms, treatment and complicating diseases, clinical examination, a one minute ECG and a chest X-ray. The information thus obtained was supplemented by existing material from admissions in our or other departments during the period under observation.

The χ^2 test was employed for the statistical analysis. A significance level of 0.05 was chosen.

Abbreviations: SND = sinus node dysfunction, ECG = electrocardiogram.

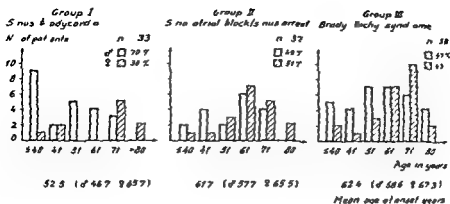


Fig 1 Distribution of age and ECO class, 128 patients at the onset of symptoms

RESULTS

Fig 1 shows the age at the time of the onset of symptoms or signs for men and women in the three groups. All age groups were represented. The male:female ratio was almost 1:1 in groups II and III, while males predominated in group I due to an accumulation of younger men.

The average age at the time of onset was highest in women and similar in all three groups. A bimodal distribution was seen for men in group I which produced a lower average age at the onset than in groups II and III.

The symptoms lasted for up to 40 years, longest in group III. Patients with symptoms of only a few days' duration resulting in hospitalization were however found in all groups.

Heart disease was demonstrated in two thirds of the patients (Table 1). Ischaemic heart disease was most frequently seen and diagnosed in 56% of all patients. Further it occurred with a significantly higher frequency in group III than in groups I and II. Conduction disturbances other than SND were noted in one third of the patients. Only first degree AV block (15 patients) and left anterior hemiblock (27 patients) were of quantitative importance.

Like in earlier investigations, cerebral symptoms (syncope, dizziness) were the main causes of hospitalization (Table II). Four patients were free from symptoms. A considerable number of patients in group III complained of attacks of palpitation or tachycardia. Congestive heart failure was not the major problem in any case. The frequency of cerebral symptoms increased from group I to group III, but the difference is not statistically significant. Almost all attacks of syncope in group I were of brief duration and rare, whereas they were more frequent and prolonged in the other two groups.

Medical treatment was attempted in all with symptoms due to SND. In cases of bradycardia and block, atropine and isoprenaline were administered, while digoxin, often in combination with quinidine and/or β receptor blocking, was given in cases of tachycardia. However, medical treatment alone was successful in a few only (Table II). Thus, only nine of 44 patients in group III did well on medical treatment. Further therapy was given in cases with severe symptoms when medical treatment had failed. This was the case in one patient in group I, in 38% of the patients in group II and 62% in group III.

Table 1 Heart disease and associated conduction disturbances in the three groups of patients with SND

LAH = left anterior hemiblock RBBB = right bundle branch block LBBB = left bundle branch block

	Group I		Group II		Group III	
	n	%	n	%	n	%
Heart disease						
Ischaemic	14	42	18	49	40	69
Rheumatic					3	
Hypertensive	3		2		13	
No heart disease	16		17			
Associated conduction disturbances						
1st degree AV block	5		3		7	
2nd degree AV block					3	
LAH	4		11		17	
LBBB			1		1	
RBBB			3		4	
RBBB + LAH	3		1			
No conduction disturbances	22		22		37	

II Symptoms and treatment in 128 patients with SND

symptoms when admitted to hospital and treatment when dismissed B = symptoms and treatment at follow up
95% confidence limits ($p < 0.05$) are indicated in parentheses

	Group I		Group II		Group III	
	A (n=33)	B (n=29)	A (n=37)	B (n=31)	A (n=58)	B (n=44)
syncope attacks	11 33% (18-52)	4 14% (4-32)	17 45% (29-62)	1* (0-17)	34 59% (45-71)	4* 9% (3-22)
bradycardia	12 36% (11-55)	12 41% (24-61)	24 63% (47-80)	14 45% (27-64)	33 57% (43-70)	10* 23% (11-38)
need of fast or brady heart therapy	11 33% (11-55)	7 24% (11-38)	4 11% (4-24)	7 23% (11-38)	15 26% (17-39)	16 36% (24-50)
pacemaker	1 3% (0-11)	2 7% (0-16)	13 35% (21-50)	1* 3% (0-11)	36 62% (50-74)	28 64% (50-74)
drugs	7 21% (11-33)	6 21% (11-33)	6 16% (8-24)	4 13% (6-20)	9 16% (10-22)	7 16% (10-22)
total	25 76% (65-90)	21 72% (56-88)	17 46% (33-59)	14 45% (27-64)	13 22% (13-30)	9 21% (11-38)

* compared to results in column A
† patients also treated with drugs

up
patients died and eight absented themselves
the follow up examination thus including 104
s (Table III). The average period of obser-
was approximately three years but in 10%
cases 9-12 months only.

of SND in addition to sinus bradycardia
noted in six patients originally belonging to
I. Two patients showed episodes of sinoatrial
and four had developed supraventricular
ardia (three atrial fibrillation one paroxys-
tachycardia the character of which could not
be ascertained).

group II three patients showed a changed
I (one atrial fibrillation two paroxysmal
ardia).

There were nine cases of probable or proven
embolism. Eight patients had had a stroke
sudden onset. Five of them had died. One pa-
tient had an embolus in the femoral artery pre-
ceded by atrial fibrillation. These nine patients had
tachy syndrome.

Deaths

Of the 16 deaths occurred in group II during the
follow up examination (autopsy findings amyloidosis
heart pulmonary embolism). Table IV shows
causes of death and the autopsy findings in the
patients who died during the period of observa-
tion. Pacemaker dysfunction was not observed

In one case a 55 year old man death could have
been caused by SND. He had had severe angina
pectoris and brady tachy syndrome as well as ear-
lier attacks of syncope. He died suddenly without
prior symptoms of myocardial infarction. Autopsy
was not carried out.

Symptoms and treatment

Approximately one third of the patients were free
from symptoms at the follow up examination (Table
II). Sixteen of these were treated with a pacemaker

Table III Follow up examination in 104 patients
with SND

	Group		
	I	II	III
No. of pats	29	31	44
No. of deaths	1	5	10
No. of drop-outs	3	1	4
Mean age (y)	57	64	68
Observation period (months)			
Mean	39	34	29
Range	9-95	9-68	9-64
No. of pats with pro- gressive SND	6	3	
No. of pats with possible systemic embolism	1	1	8
No. of pats treated for congestive heart failure	5 (17%)	7 (23%)	15 (34%)

Table IV *Deaths during the observation period*

	Sex	Age (y)	Months between discharge and death	Pace maker treatment	Cause of death	Autopsy performed
Group I	♀	86	6		Pneumonia	
Group II	♂	64	26	+	Brain tumour	-
	♂	81	22	+	Bleeding peptic ulcer	
	♀	89	18		Pulmonary embolism	+
Group III	♂	78	10	+	Respiratory failure due to chronic bronchitis	+
	♂	84	13	+	Congestive heart failure recent MI	
	♂	62	50		Brain abscess?	
	♀	81	4		Pneumonia cerebral apoplexy	
	♀	85	17	+	Pulmonary embolism cerebral apoplexy	+
	♂	55	3		Cardiac arrest (asystole?)	
	♀	86	34	+	Pneumonia cerebral apoplexy	+
	♀	71	11	+	Gangrene of the intestine	+ incomplete
	♂	69	16		Pulmonary embolism cerebral apoplexy	+
	♂	72	8		Cerebral apoplexy	

Nine patients had syncope during the observation period. One of these had a pacemaker; there were no signs of pacemaker dysfunction but severe cerebral arteriosclerosis to which the symptoms were attributed. Also in five others the syncope was considered to have been caused by a cerebral disease (epilepsy in one, cerebral arteriosclerosis in four). One patient with syncope due to SND had been prescribed a pacemaker but this kind of treatment had not been given. In the remaining two patients with syncope during the observation period the follow-up revealed SND as the cause. This relationship had been overlooked during the primary admission. In both patients the syncope ceased after pacemaker implantation.

In 11 patients not treated with pacemaker during the primary admission despite a history of syncope, no such attacks occurred during the observation period.

Table II shows that supplementary antirhythmic treatment had been necessary during the observation period in the majority of patients treated with a pacemaker in group III. The number of patients who managed without treatment was unchanged in all three groups.

DISCUSSION

The diagnostic criteria for SND have varied somewhat in the literature. Ferrer (7) suggested a division into six groups based on ECG criteria.

Included in these were patients with chronic fibrillation and cases with prolonged asystole or bradycardia after DC conversion. These two are not considered in our investigation as they of necessity include patients who do not suffer from SND as defined today. The remaining four correspond to our groups I and II. However, brady-tachy syndrome has now been recognized as a characteristic manifestation of SND. To this we have employed the criteria of Rubenstein (16). Although based on easily recognized criteria, this grouping gives a clinically important distinction between types of bradycardia and brady-tachy syndrome, in contrast to electrophysiological criteria, the use of which is not generally accepted (3, 11, 14).

Only a few reports covering SND in a patient series have been published. Since 1960 occurred in 70% of the 51 patients reported by Rokseth and Hatle (15) and of those 71 reported by Rasmussen (13) as there were relatively few patients with brady-tachy syndrome. Pacemaker treatment was employed in 45 and 81% respectively. Eraut and Shaw (5) described 46 patients, of whom half had disturbances of consciousness. The majority of these patients had sinus bradycardia; pacemaker treatment was given in one case only. A direct comparison between our patients and those mentioned is therefore impossible due to differences in composition.

In the study of 56 patients reported by B

al (16) there are certain similarities to the investigation. This applies in particular to age of the patients and the occurrence of the disease (45 and 48% respectively). The frequency is almost 1:1 in the majority of rubenstein et al found a slight predominance of women while the present work includes men. This can be attributed to an accumulation of younger men with sinus bradycardia which has an apparent bimodal distribution of the onset in group I. The work of Rubenstein suggests the same and thus the question arises whether these younger men differ from the whole group in actual fact do not suffer from SND.

Dispersion with regard to the time of onset is small but the average age at the time of onset in women was similar in all groups and for men in groups II and III (Fig. 1). This supports the assumption that the progression from sinus bradycardia to a brady-tachy syndrome is a common phenomenon as the average age at onset was almost the same in all three groups. For such a course is known and has been reported in nine of our patients (Table III) at the time of onset was on the whole about ten years higher in women than in men, an aspect which is somewhat similar to the situation for ischaemic heart disease.

The etiology of SND is unknown and different causes may be of importance. Hereditary occurrence has been reported with manifestation already in childhood or early youth (9). One third of our patients had no signs of other heart disease. Ischaemic heart disease was demonstrated in more than half of the patients while considerably lower frequencies were found in earlier publications 35% (14%) (15). The fact that SND can occur in myocardial infarction seems to show that this may be a factor of importance. Ischaemic heart disease was demonstrated significantly more frequently among our patients in group III than in groups I and II. This raises the question as to whether tachycardias in this group originate from the parts of the atrium. However patho-anatomical and angiographic investigations do not come to a definite conclusion on this point (4, 10).

One of our patients had a pacemaker. Pacemaker treatment was not considered indicated in 13 of our patients even though they had suffered from at least one syncope. All but two patients were free

from such attacks during the period of observation. Their syncope subsided after pacemaker implantation. Thus these two patients have possibly been the object of erroneous primary evaluation. It should in addition be mentioned that asystolia caused by SND may have been the cause of death in one case but sudden death (ventricular fibrillation) due to ischaemic heart disease is the most likely explanation.

It may be concluded that the routine programme of the department has functioned effectively. This includes telemetric observation during hospitalization and a documented relationship between the symptoms and SND prior to the decision to implant a pacemaker. However prolonged observation is necessary in a number of patients as the symptoms are often intermittent and for this reason ambulatory monitoring using the technique of Holter must be considered advantageous (14).

On the basis of the present investigation it is impossible to determine whether pacemaker treatment has been employed too frequently. Pacemaker treatment has been given in other investigations with a similar frequency provided correction is made for the difference in the composition of the patient series.

The follow-up examination has shown that supplementary antiarrhythmic treatment within a few years after implantation of a pacemaker is required in the majority of patients in group III. This should be borne in mind during the control examination following pacemaker implantation.

Nine cases of possible systemic embolism were observed during the observation time of approximately three years. In eight cases this took the form of a stroke and five were fatal. All of these patients had brady-tachy syndrome. This is in agreement with previous investigations (1, 6, 12). None of our patients were on anticoagulant therapy. However it could be an important aid in reducing the fatalities as one half of the deaths in group III were caused by apoplectic insults or complications to such. Apart from the increased frequency of embolic phenomena in group III, SND appears in general to have a good prognosis when the usual treatment is given. Several patients had recurring attacks of syncope for some years without any sequelae. So the prognosis for untreated patients with syncope due to SND seems to be much better than for untreated patients with syncope due to atrioventricular block.

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The Diagnosis of Atrial Myxomas

Difficulties and Pitfalls

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ABSTRACT Altogether seven patients with the preoperative diagnosis of atrial myxoma were admitted to our hospital in 1968-77 and referred for surgery. In two patients no tumour was found at operation. In one of them the reason for the misdiagnosis was severely malformed mitral leaflets. In the other the contrast medium was injected into a coronary vein which caused a contrast defect due to inadequate mixing.

Key words: myxoma, diagnosis.
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Atrial myxoma is the most common type of the rare group of primary heart tumours. Three fourths of all myxomas are located in the left atrium, most of the remaining ones in the right (5). Since the tumour is often asymptomatic and its symptoms are variable, the diagnosis is easily overlooked or delayed (4). Although myxomas are rare, it is essential that they are diagnosed and removed since they involve risks of serious complications. A definite preoperative diagnosis of myxoma of the heart is established by means of echocardiography and/or cardioangiography. In our experience, however, these methods entail limitations which in some instances even leading to erroneous cardiac surgery. This study describes some of the factors behind the misinterpretation of the angiographical and echocardiographical findings of atrial myxomas, based on findings in myxoma patients.

PATIENTS AND METHODS

In 1968-78 seven patients with the preoperative diagnosis of myxoma of the atrium were referred for surgery at Sahlgren's Hospital in Gothenburg. Six of them had a myxoma in the left atrium, one in the right. At the time of diagnosis, the diagnoses were confirmed in only five pa-

tients. The most important findings are summarized in the following case reports.

Case I

A 36-year-old man, acutely ill with right-sided paralysis, ESR 12 mm. Carotid angiography: occlusion of the medial cerebral artery. Echocardiography (Fig. 1) as well as cardioangiography ten months after the onset of symptoms showed typical pictures of tumour in the left atrium extending down through the mitral valve in diastole. Operation: myxoma of the left atrium. Mitral valve leaflets normal.

Case II

A 36-year-old woman with no previous heart symptoms. Gradually taken ill with frequent fever attacks, often accompanied by pain in the right hypochondrium, increasing tiredness and exertional dyspnoea. Because of the confusing abdominal pains, laparotomy was performed about one year after the onset of symptoms, but only a non-specific enlargement of the liver was found. A few weeks later additional chest pains were noted, as well as congestion of the veins of the neck and a murmur to the left of the sternum. ESR 60 mm. Chest X-ray now showed a general enlargement of the heart. The new cardiac findings led to cardioangiography, disclosing a tumour of the right atrium. Operation 12 months after the onset of symptoms: myxoma of the right atrium.

Case III

A 40-year-old previously healthy woman, fallen ill with rapidly progressing tiredness and increasing dyspnoea. ESR 3-6 mm. Roentgenologic and auscultatory findings compatible with mitral stenosis. Echocardiography and cardioangiography revealed a tumour of the left atrium, partly protruding into the left ventricle in diastole. Operation eight months after the onset of symptoms: myxoma of the left atrium.

Case IV

A 64-year-old previously healthy woman, fallen acutely ill with saddle embolus. Embolic specimen contained myxomatous tissue. Echocardiography immediately after embolectomy was negative, but a second echocardiography eight months later showed that the size of the left atrium had decreased from 4.5 to 2.8 cm, but there were no echoes suggestive of myxoma. ESR 6 mm. Subsequent



Fig 1 Case I Scanning from the left ventricle to the mitral valve showing classic stratified echoes of left atrial myxoma

cardioangiography however revealed a tumour of the left atrium not in contact with the mitral valve leaflets. Operation eight months after the embolic incident. Myxoma. Mitral leaflets normal.

Case 6

A 65 year-old woman with no previous cardiac disease. During ten months increasing dyspnoea and cardiac dysfunction down to function group IV. ESR 19-32 mm. Auscultation and chest X ray indicative of mitral stenosis however both echocardiography and cardioangiography showed pictures typical of tumour of the left atrium partly obstructing the mitral orifice. Operation ten months after the onset of symptoms. Myxoma. Mitral leaflets normal.

Case 7

A 12 year-old boy healthy until the age of seven. Thereafter repeated attacks of unconsciousness sometimes in connection with ventricular extrasystoles and on one

occasion transient cardiac arrest. ESR 2 mm. Aetiology of these attacks was unclear. It was not necessary to rule out the possibility of cardiac disease. Echocardiography of the left atrium was abnormal though the picture was not quite typical of myxoma. Monary angiogram showed a normal left atrium. Subsequent contrast injection directly into the left atrium resulted in contrast defects suggestive of tumour. Normal left atrium.

Case 8

A 32 year old woman with heart murmur known for years. Admitted because of increasing cardiac dyspnoea. ESR 26 mm. Auscultation and chest X ray suggested mitral stenosis. Echocardiography showed a tumour of the left atrium (Fig. 2) but cardioangiography did not show any contrast defects. Operation revealed a congenitally deformed and thickened mitral valve and atrial tumour.

Non-invasive studies of the heart including echocardiography, pulse curves, apexcardiography and cineangiography were performed in all patients at the Department of Clinical Physiology with the exception of Case 1 as the above methods were not yet in practice in 1968 (7).

The cardioangiographies were performed by contrast injection into either the right atrium or the pulmonary artery depending on the expected location of the tumour. In case 4 a contrast injection into the left atrium was made in addition to the injection into the pulmonary artery.

RESULTS AND DISCUSSION

Atrial myxomas were found at operation in 8 cases—four on the left side and one on the right. In the remaining two patients, one had congenitally malformed stenotic mitral valves whereas the other was normal.

The clinical symptoms and findings had been further examination and surgery were normal.

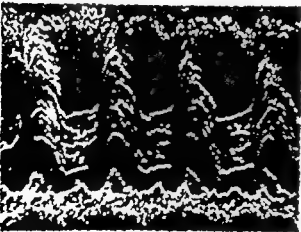
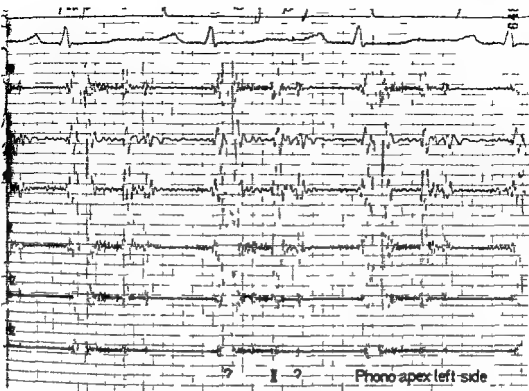


Fig 2 Case VII Polaroid photo showing parallel stratified echoes suggestive of left atrial myxoma. A congenitally deformed stenotic mitral valve was however found at surgery.



Case III Phonocardiogram showing a very strong S1 sound and a diastolic extra sound. The question

marks here indicate that these sounds are not typical of mitral stenosis (see Fig. 4)

valvular disease in three patients (III, V and VI) peripheral artery emboli without any clinical evidence of heart disease in two (I and IV). In one (II) puzzling symptoms in combination with heart failure were noted. One patient (VI) had uncharacteristic disturbances of consciousness caused by arrhythmias.

In one of the verified myxomas was suspected on clinical grounds but only after symptoms had been present for ten months (case I). For none of the patients showed any classic risk symptoms indicative of myxoma such as combination of positional dyspnoea, long attacks of fever and high ESR (5). The only patient with typical positional dyspnoea was the one with mitral stenosis (case VII).

The most confusing clinical picture was produced by right atrial myxoma. Because of unexplained peripheral oedema with ascites an exploratory laparotomy was performed in this case. Suspicion was directed towards the heart until the patient died of chest pain and increasing distension of the neck. The complex symptomatology

of right atrium myxomas however is well known and has been described earlier (5).

In no patient was the final diagnosis made earlier than eight months after the onset of symptoms. In two (III and V) suspicion of myxoma arose as a result of a routine echocardiographic examination for possible mitral stenosis. The remaining two myxomas (I and IV) which presented clinically as emboli were revealed only because the source of the emboli was searched for.

In three cases (I, III and V) pictures typical of myxoma of the left atrium were found by non invasive means and by angiography. The characteristic picture with parallel echoes behind the mitral leaflets was seen on echocardiography (Fig. 1). These patients had also a quite clear diastolic extra sound differing from an opening snap by its unusual low frequency and which when registered together with the apex curve appeared clearly after the Q-point and sometimes even after the rapid filling wave i.e. too late to be an opening snap (Figs. 3 and 4). All these patients also displayed a very strong and extraordinarily late component in their first heart

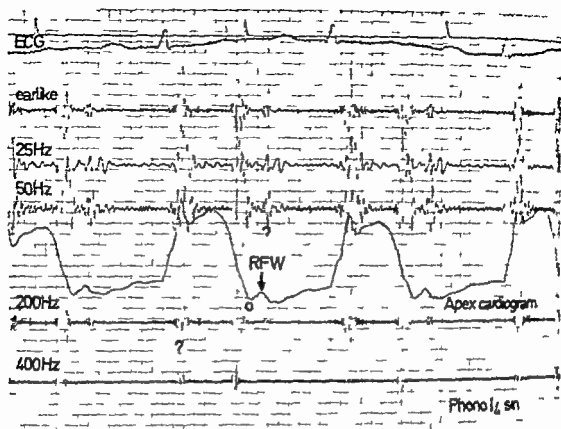


Fig 4 Case III Apexcardiography shows that the first heart sound is simultaneous with the top of the apexcardiogram and that the diastolic extra sound comes later than the rapid filling wave (RFW). These time relations

exclude the possibility of mitral stenosis and suggest the sounds are produced by atrial myxoma, prior to the sudden arrest in the swinging movement.

sound far too late to be a normal mitral closing sound and probably like the early diastolic extra sound produced by movements of the myxoma (Figs 3 and 4) (1, 2).

Echocardiography immediately after the embolic episode was negative in case II. At repeated examination eight months later the size of the left atrium had decreased from 4.5 to 2.8 cm. Whether this reduction in atrial size was due to regeneration of a myxomatous tumour or a technical error could not be decided. The absence of echocardiographic findings typical of myxoma may be explained by the myxoma not reaching down to the mitral valves. The echocardiogram in case II in whom operation showed a malformed mitral valve could not be differentiated from that of a left atrial myxoma (Fig 2). However at the time of these examinations (cases IV and VII) the echoes were registered by means of polaroid photos from a storage oscilloscope. Later technical developments such as gray scale recording and scanning have improved the

diagnostic accuracy of echocardiography considerably (Fig 1). Nevertheless the extra swinging at the place of an ordinary opening would have made us more reluctant to accept diagnosis of myxoma in case III (see also diffuse immobile unstratified cloud of echo in the left atrium) i.e. findings not typical of myxoma could be registered in case VI by means of a chart recorder as well as by two-dimensional echocardiography. However due to the very serious history of this patient with stroke and cardiac arrest additional cardiac surgery was considered necessary.

Since 1978 we include two-dimensional scan echocardiography in our non-invasive investigations. This technique is superior in detecting masses in the heart and probably will solve the diagnostic problems in cases IV, VI and VII.

All patients with surgical verification were correctly diagnosed by means of echocardiography. In patient VII with severely malformed mitral

where the echo picture suggested myxoma the angiography was negative with regard to tumour. Only angiographically misinterpreted case. The left atrium appeared normal at pulmonary angiography. At an additional contrast injection in the left atrium the tip of the catheter was erroneously placed in a pulmonary vein which thereby contributed to the appearance of a filling defect in the atrium erroneously interpreted as a tumour. At operation the left atrium was normal. Collectively it appears that the angiographical contrast defect must have been caused by inadequate mixing of contrast medium in the atrium due to the position of the catheter. (6) A correct diagnosis would have been avoided if the contrast had been made directly into the left atrium or only a pulmonary angiography had been performed.

Thus when a tumour in the left atrium is suspected a contrast injection into the pulmonary artery is the method of choice. It is advisable to use the cine technique since this often allows the observation of the typical movements of the tumour as opposed to mural thrombi (5).

CONCLUSIONS

There is no clinical picture which can be regarded as typical of atrial myxoma. The clinical findings may primarily raise suspicions of myxoma in the presence of mitral stenosis often with remarkably rapid progression and second peripheral emboli especially in young and otherwise healthy individuals.

Echocardiography must be performed in all cases of suspected mitral stenosis since echocardiography combined with phonocardiography and apexcardiography will give a conclusive diagnosis of all cases causing obstruction of the mitral valves.

(1-2) Confirming angiography should be unnecessary in such cases. Echocardiographic findings that are difficult to interpret and cases of negative echocardiograms should when the clinical evidence is strongly suggestive of myxoma be followed by angiography. Surgically removed peripheral emboli must be examined microscopically for myxomatous tissue.

Myxoma is a rare cause of arterial embolization. All patients with this symptom cannot be examined with regard to cardiac tumour. However an unexplained peripheral embolus in a young person should suggest myxoma (5). Since echocardiography and probably angiography immediately after an embolization may give negative results (case IV) such examinations should be repeated after 6-12 months in cases with clinical findings strongly suggestive of myxoma (3).

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The Clinical Impact of Long-Term ECG Recording

A Retrospective Study of 150 Patients

Leif Eriksson and Olle Pahlm

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ACT One hundred and fifty patients referred ECG (LECG) recording at a university were monitored for 12 hours or more. The physicians' patient records were studied 12 more after monitoring in an attempt to find how LECG had affected patient management. Seventeen patients were treated with pacemakers and 13 with antiarrhythmic drugs as a direct result of LECG. Thirteen patients entered symptoms with concomitant cardiac arrhythmia at the time of recording were considered for treatment. In 17 patients who experienced symptoms without concomitant arrhythmia monitoring cardiac arrhythmia could be established as the cause of the symptoms. In 9 more, LECG was considered to have contributed to the clinical information (which could not be obtained by other diagnostic methods) to the physician. Thus LECG was considered to have affected the referring physician's management decisions in 69 cases (46%).

Key words: arrhythmia, diagnosis, arrhythmia, therapy, ambulatory tape recording.
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Long-term ECG (LECG) monitoring using a portable recorder is a well established diagnostic methodology (1-3, 4). Although the application of this technique are manifold, two areas are dominant: 1) Follow-up of patients with prior myocardial infarction (MI) in order to find individual risk of sudden cardiac death; 2) Investigate patients with symptoms possibly caused by intermittent cardiac arrhythmia. The relationship between severe ventricular arrhythmia and subsequent sudden death in the MI period has been demonstrated (11, 14). However, application has not attracted much attention in Sweden. The lack of evidence that treatment of ventricular arrhythmia in these patients re-

duces the risk of sudden death may account for the reluctant attitude of the cardiologists. The value of LECG in the management of the latter category of patients is more evident and has been demonstrated by many authors (7, 9, 12). If one succeeds in recording the LECG during symptoms of the kind that the patient has complained of cardiac arrhythmia can either be verified or excluded as the cause of the patient's complaints. In the former case therapeutic intervention (pacemaker therapy, antiarrhythmic drugs) often relieves the patient of his symptoms. In the latter case unnecessary therapy may be avoided and further investigation can be conducted to other directions. However, useful clinical information can sometimes be obtained from LECG even if the patient had no symptoms at the time of recording.

In an attempt to evaluate the impact of LECG on patient management we studied 150 patients retrospectively.

STUDY POPULATION

A total of 150 consecutive patients referred from seven departments in our hospital (Table I) during the period Aug 1976-Oct 1977 were included in the study. Their mean age was 59 years, 57% were males, 50% were referred because of symptoms of possible cardiac origin (Table II). Most of the recordings were made on outpatients.

METHODS

Each patient was monitored for at least 12 hours using a portable one-channel FM cassette tape recorder (SPA 441-201). A detailed diary of activities and symptoms was required. A small number of patients were monitored for more than 12 hours, 36 hours being the maximum. Twenty-one patients were subjected to repeated monitoring periods, 11 or 3 separate occasions.

Abbreviations: LECG=long-term electrocardiogram; MI=myocardial infarction.

Table I Number of patients from the seven referring departments

Referring department	No. of pats
Cardiology	60
Internal Medicine	45
Neurology	22
Thoracic Surgery	12
Pediatric Cardiology	9
Pulmonary Medicine	1
Urology	1
Total	150

The tapes were analyzed by a computer aided method (10). We studied the referring departments' patient records and our diagnostic reports 12 months or more after the recording in an attempt to establish if and how LECG had affected the management of the patients. To that end each patient was assigned to one of the following six categories: 1) The LECG led to implantation of a permanent pacemaker. 2) The LECG led to initiation of or change in antiarrhythmic drug therapy. 3) Arrhythmia was found on the LECG during periods of relevant symptoms but the patient received no treatment as a result of the findings. (The findings at LECG were nevertheless regarded as valuable since a diagnosis could be made.) 4) No arrhythmia was found during periods of relevant symptoms; cardiac arrhythmia could therefore be excluded as the cause of the patient's complaints. 5) Other cases in which the result of LECG information was judged to be valuable for the referring physician. 6) No evidence that the result of LECG provided valuable information to the referring physician.

RESULTS

Out of the 150 patients under study, 135 were referred because of symptoms which might be due to cardiac arrhythmia (Table II). Relevant symptoms during the time of recording were experienced by 54 of them. LECG was conclusive in 44 of these patients; cardiac arrhythmia could either be verified (27 patients) or excluded (17 patients) as the cause of the symptoms. In 10 patients there was a doubtful relationship between reported symptoms and detected arrhythmia.

Altogether 69 patients (46%) 44 of them with relevant symptoms during LECG and 25 without such symptoms were considered to belong to categories 1-5 in which LECG to some extent contributed to patient management (Table III).

Pacemaker therapy

A permanent pacemaker was implanted in 17 patients as a direct result of LECG: in 15 due to sinus

node dysfunction in 2 due to a permanent conduction defects. Six of these 17 patients experienced symptoms during the monitored period: bradycardia.

Antiarrhythmic drug therapy

Antiarrhythmic drugs were administered to 16 patients as a direct result of findings of tachyarrhythmia on the LECG. Eight of these 16 patients experienced symptoms during the monitored period: tachyarrhythmia. Six of these patients were given digitalis, three verapamil, two ß-blockers, one quinidine and one procainamide.

Symptoms and arrhythmia but no treatment

Altogether 27 patients had cardiac arrhythmia during symptoms. In 13 of them no treatment was instituted as a result of the findings. Six of these 13 patients had a subjective feeling of tachyarrhythmia. The objective finding of tachyarrhythmia as the cause of the symptoms was considered to have a reassuring influence on the patients and the findings at LECG were considered useful.

Symptoms but no arrhythmia

Seventeen patients experienced symptoms but no concomitant arrhythmia during LECG investigation led to a neurological diagnosis in 10 patients. In 3 patients a psychogenic cause was considered likely. In the remaining 4 patients the cause of the symptoms was still unknown at the end of follow-up.

Table II Indication for LECG recording in 150 non-selected patients

Indication	No.
Patient suffers from symptoms which might be due to cardiac arrhythmia	135
Syncope	4
Dizziness "feeling faint"	2
Palpitations, irregular heart rhythm etc.	7
Chest pain	3
Other symptoms	6

Patient does not suffer from any symptoms

Check-up of frequency and/or severity of arrhythmia already by resting ECG

Total

study points to the value of analyzing also the tapes from patients without symptoms during the time of recording

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The Outcome of Patients with Transient Ischemic Attacks and Stroke Treated with Anticoagulants

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CT Long-term anticoagulant treatment in 1125 patients with transient ischemic attack (TIA) and 49 stroke patients with reversible neurological deficit or cerebral embolism. 16 TIA patients were observed without anti-

Life table analyses, comparing observed with the expected frequency, revealed mortality in the TIA patients irrespective of whether or not they had received anticoagulant treatment. In the stroke patients treated with anticoagulants, a higher mortality than expected was observed. On the other hand, the incidence of subsequent stroke was lower than expected in the TIA and stroke patients treated with anticoagulants, while it was significantly increased in the TIA patients not treated with anticoagulants. Thus the risk of stroke, but not the death rate, was normalized by the anticoagulant treatment. Unacceptably serious bleeding complications were seen in the group of stroke patients with anticoagulant treatment. Bleeding complications in TIA and stroke patients seemed to be related to treatment, high blood pressure or administration of treatment. High patient compliance

by the Joint Committee for Stroke Facilities in 1973 (18). The present report deals with the influence of the therapy on the survival time and the frequency of new cerebrovascular symptoms in these patients. The side effects observed during anticoagulant treatment are also reported.

PATIENTS AND METHODS

The patients are the 74 who received anticoagulant treatment during a 4-year epidemiological study of TIA and stroke in the community of Söderhamn starting in 1975. The method for tracing and registration has been reported elsewhere (19). Forty-four patients with debuting TIA were registered. 8 of them were considered to have no general contraindications for anticoagulant therapy. Three of these patients underwent neck vessel surgery and were excluded, leaving 25 patients in the TIA anticoagulant group. The remaining 16 TIA patients received the standard therapy given to all patients when necessary for diseases other than cerebrovascular, but no antiplatelet aggregating agents or vasodilators were administered.

Forty-nine patients with stroke were treated with anticoagulants. They were a minority of the 111 patients who had their first stroke during this period. TIA is defined as rapidly developed clinical signs of focal cerebral dysfunction of presumed vascular origin, not lasting more than 24 hours. Drop attacks or vertigo without any other symptoms, as well as migraine with associated neurological symptoms, were not sufficient for the diagnosis of TIA. TIA recurrence implies a new attack after a symptom-free interval of more than 24 hours. The stroke patients belonged to one of two categories: 34 suspected of embolization from the heart or large vessels having minimal residual symptoms, and 15 with reversible ischemic neurological deficit (RIND). In the latter category, the symptoms subsequently disappeared but after more than 24 hours.

The following numbers in the Minnesota code (3) were

Abbreviations: TIA, transient ischemic attack; RIND, reversible ischemic neurological deficit; TT, Thrombotest®; BP, blood pressure.

for anticoagulant treatment, transient ischemic attack, stroke, complications.

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Anticoagulant treatment for the prevention of cerebrovascular disease was first reported by Muller in 1955 (16). Since then, alternative anticoagulant treatment with antiplatelet aggregating agents has been presented (8). Though definite lack of evidence on the protective role of all these agents, 6-9) anticoagulants are still recommended for patients with transient ischemic attacks, progressing stroke or embolism of unknown origin (10, 13, 22).

In a prospective epidemiological survey of stroke, anticoagulant treatment was according to the recommendations issued

study points to the value of analyzing also the tapes from patients without symptoms during the time of recording

ACKNOWLEDGEMENTS

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d. Causes of death

Sex	Anticoagulant group	Non anticoagulant group	Stroke anticoagulant group	
			Age (yr)	Sex
1	Myocardial infarction		44	♂
	Gangrene		66	♂
1	Cancer		66	♂
1	Renal failure		67	♀
	Cancer		71	♂
	Congestive heart failure		71	♂
	Stroke		72	♀
	Stroke		72	♂
	Stroke		74	♂
	Stroke		74	♀
			76	♀
			78	♂
			79	♀
			81	♂
			84	♂
				Renal failure
				Stroke
				Congestive heart failure
				Stroke
				Stroke
				Intestinal hemorrhage
				Myocardial infarction
				Myocardial infarction
				Pneumonia
				Septicemia
				Myocardial infarction
				Cancer
				Stroke
				Pneumonia

RESULTS

Age and mean BP during the first week of observation were lower in the group of patients with anticoagulants than in the group receiving standard therapy while no difference was found in distribution by sex. The proportion of patients with definite ECG changes was not significantly higher among those given standard therapy. In the group of stroke patients treated with anticoagulants there was a male preponderance.

Observed survival curves as well as the expected curves for the expected survival in the group are shown in Figs 1-3. In the interval years of observation the mortality is higher in both TIA groups, the change being significant ($p < 0.05$) in the anticoagulant

group (Fig. 1). The cause of death was stroke related in 4 out of 6 patients in the TIA group given standard therapy while none of the 4 patients who died in the TIA anticoagulant group had suffered from a stroke (Table II). In the group of stroke patients given anticoagulant treatment the mortality was significantly increased in periods 1-2, 2-3 and 3-4 years (Fig. 3). Five of the 15 patients in the stroke anticoagulant group who died had recently suffered from a new stroke (Table II). Cardiovascular disease was the major cause of death in all groups.

The incidence of stroke was significantly increased during the interval 2-3 years in relation to the expected rate in the group of TIA patients given standard therapy (Fig. 4). On the other hand the TIA and stroke groups with anticoagulant treatment

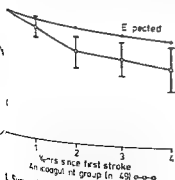


Fig. 1 Survival after the first symptoms in 49 stroke patients with anticoagulant treatment (O) compared to the expected rate in an age- and sex matched population.

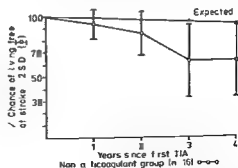


Fig. 4 The chance of living free from stroke after the first symptoms in 16 patients without anticoagulant treatment (O) compared to the expected chance in an age- and sex matched population.

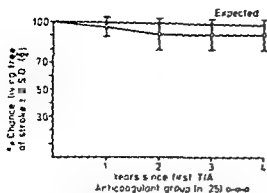


Fig. 5 The chance of living free from stroke after the first symptoms in 25 TIA patients with anticoagulant treatment (O) compared to the expected chance in an age and sex matched population

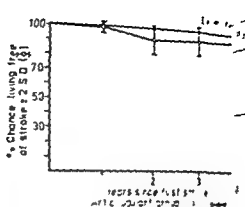


Fig. 6 The chance of living free from stroke in 49 stroke patients with anticoagulant treatment (O) compared to the expected chance of living free in an age and sex matched population

did not display any significant increase in incidence of stroke (Figs 5 and 6)

All three patients who underwent neck vessel surgery are alive and free from stroke and TIA recurrences after observation times of 19, 26 and 30 months, respectively

The proportion of patients having TIA recurrence after the initiation of anticoagulant treatment was about the same as in the standard treatment group

Table III Number of TIA patients with 1, 2-4 or more recurrences of TIA

	No. of recurrences		
	1	2-4	≥5
Anticoagulant group (n=25)	6	2	1
Non anticoagulant group (n=16)	2	3	1

Table IV Number of recurrent TIAs in relation to TT values in that period (n=25)

TT value (%)	No. of recurrences		
	1	2-4	≥5
<5*	-	-	-
5-15	5	1	-
>15*	-	1	-
Unstable*	1	-	1

* Not within the 5-15% interval in one direction twice or more yearly

† Not within the 5-15% interval in either direction twice or more yearly

(Table III) The majority of those with a TIA during anticoagulant treatment fulfilled criteria for satisfactory treatment i.e. their TT were within the range of 5-15% during 4 years in question (Table IV). The TT values were satisfactorily high at least once a year in 13 of the patients in whom TIA recurred. This figure does not differ from that in the whole group (25). One third of the TIA and stroke patients had TT values outside the therapeutic window, or more during the first year of treatment (V). The values were more stable during the 1st year when only 5 patients out of 10 had 4 values twice or more.

Fifteen bleeding complications occurred in 14 TIA and 11 in the stroke anticoagulant group (VI) making hospital care necessary. The proportion in the stroke group is not statistically significant. Nine of the complications occurred after 9 months of treatment. Four occurred

Table V Therapeutic control expressed in values in relation to duration of treatment

TT value (%)	Year of treatment			
	1st	2nd	3rd	4th
<15	49	25	13	4
<5	3	-	1	-
>15	7	-	-	-
Unstable	14	5	-	-
Total	73*	30	14	4

* One patient lost for wound treatment died sixth month

Complications during anticoagulant therapy in relation to age duration of therapy BP on admission (BP_a), mean BP in the first week (BP_w) and TT value

	Age (y)	Duration of therapy (mo)	BP	BP _w	TT value (%)	Outcome
intracranial hemorrhage	73	39	160/105	131/77	5-15	
intracranial hemorrhage	63	15	150/80	134/84	Unstable	
intracranial hemorrhage	63	3	140/60	147/62	5-15	
intracranial hemorrhage	77	2	190/85	152/76	5-15	
intracranial hemorrhage	66	9	220/105	174/95	5-15	Lethal
intracranial hemorrhage	71	13	220/135	219/117	Unstable	Lethal
intracranial hemorrhage	81	2	170/90	153/80	<5	Lethal
intracranial hemorrhage	72	2	160/120	171/92	5-15	Lethal
intracranial hemorrhage	74	34	160/120	130/89	5-15	
intracranial hemorrhage	63	39	180/90	145/81	Unstable	
intracranial hemorrhage	73	15	180/90	135/84	5-15	
intracranial hemorrhage	73	1	210/105	120/70	5-15	
intracranial hemorrhage	77	10	200/90	151/70	Unstable	
intracranial hemorrhage	70	1	150/90	138/79	<5	
intracranial hemorrhage	72	32	150/90	166/95	<5	

BP_w means were given after the BP was more adequately controlled (mean 177/97) in the second week

fatal outcome 3 intracranial hemorrhages
retinal bleeding all in the stroke group
3 patients with intracranial bleeding 2 had
diastolic BP of 95 mmHg or more while
1 had TT values of less than 5% on two
occasions during the 2 months of therapy In both
treatment groups 6 patients had a mean diastol
ic BP of 95 or more Three of these developed
complications though the BP was lowered
with hypertensive agents within 2 weeks The
diastolic BP on admission exceeded 95 in 25 of
patients given anticoagulants later on of whom
developed bleeding complications (Table VI)

DISCUSSION

In this study there was a selection of the patients
given anticoagulants in both the TIA and the stroke
groups. Consequently the patients given antico-
agulants and those given standard therapy are not
comparable with respect to age and BP distribution.
The table method enables one to compare the
observed mortality in the patient group with the
expected mortality in a population with the same
distribution by age and sex (7). Obviously the mor-
tality was increased in relation to the expected rate
in both patient groups receiving anticoagulants
in accordance with the results of 13 other

studies on the effect of anticoagulants on TIA and
stroke in which no benefit was derived in terms
of normalized mortality (10).

The comparison of observed and expected inci-
dence of stroke using the same method as described
above is probably more adequate as the refer-
ence figures are derived from the Soderhamn popu-
lation. Thus the observed normal stroke incidence
in the TIA anticoagulant group and the increased
incidence in the TIA standard treatment group
might represent a true reduction as a result of the
anticoagulant treatment since age differences are
taken into account. The other variable that differs
between these two groups is BP though the dis-
crepancy is rather small. Previous studies on the
natural course of TIA failed to show that stroke was
more likely if the patient was hypertensive (5). Thus
the present small difference is probably of marginal
importance. However the present type of compar-
isons can just indicate but never prove a benefi-
cial influence of anticoagulant treatment as little
as other types of non randomised studies (5, 6, 10).
Even harder to judge are the results of studies
where the observed incidence of stroke in the treat-
ment group is compared to the incidence in the
same patients after termination of therapy or to the
incidence in another untreated population (14, 17).
In the former case the almost exponentially in

creasing risk of stroke with increasing age as well as possible rebound effects of withdrawal of the drug are not taken into account. In the latter respect the differences between two populations regarding the variables mentioned above including heart disease make comparisons meaningless.

The patients in this study continued having TIAs during the therapy in spite of TT values mostly in the range 5–15%. The literature includes some reports of ceased TIA after initiation of anticoagulant therapy as well as increased TIA incidence after withdrawal of therapy (10). In most studies the further course of TIA after anticoagulant treatment is incompletely reported.

The complications of anticoagulant treatment in this study are rather harmless in the TIA group while the types of complications in the stroke group are unacceptably serious. There is some controversy regarding anticoagulant treatment in patients with completed stroke. Bleeding complications and even increased mortality in connection with anticoagulant treatment were reported early on (1, 2, 12). Other studies failed to show a long lasting therapeutic reduction of neurological symptoms. It was concluded that the dangers might outweigh the benefits (4, 21). The relationships between complications and BP, patient compliance and duration of treatment were however not clarified. In a recently published study of comparatively young patients with a mean treatment time of 11 months complications were acceptably few and harmless (14). The patients in that study were carefully selected and all were investigated with angiography and repeated lumbar punctures. In the present study it is notable that 3 out of the 11 patients whose mean diastolic BP was 95 or more in the first week after admission developed bleeding complications of which two intracerebral hemorrhages were lethal. This occurred despite lowering of the BP within 2 weeks during antihypertensive drug therapy. While increased mean diastolic BP is unfavorable for the outcome, the first diastolic BP after admission is hardly predictive. Most bleeding complications occurred after 9 months of treatment and were not attributable to hypertension or insufficient patient compliance.

The majority of patients with TIA or stroke seen in medical wards in Sweden are older than those who participated in most previous studies (10, 14). The results of the present study comprising all patients with onset of TIA during a 2-year period and

some old stroke patients indicate a beneficial effect of anticoagulant treatment as compared with the normal incidence of stroke. However, anticoagulants must be restricted to patients with good cooperation and low mean diastolic BP. Anticoagulant treatment should be confined to the period of greatest risk of suffering a stroke, which is 2–3 years after the onset of TIA (4) in view of the small number of unexpected late bleedings.

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Comparison of Energy and Nutrient Intakes in Women with High and Low Blood Pressure Levels

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ABSTRACT The present study found no link between intake of energy and various nutrients, on the one hand and high or low blood pressure (BP) on the other. Sixty women not on treatment for hypertension were selected from a defined population and examined applying the duplicate portion technique with respect to the relationships between the intake of energy and nutrients. They were divided into two groups: group A (group 1) from above the 95th percentile for BP (group 1) and group B (group 2) from below the 30th percentile for BP (group 2). The two groups were age-matched. The food sampling consisted of three periods of two days each, divided into three periods of two days each within a period of four weeks. Twenty-four urine specimens were collected in each period and on two other occasions. The mean values for energy, fat, protein, carbohydrates, minerals and electrolytes did not differ between the two groups despite the large differences in BP and obesity. The mean values for urinary excretion of minerals and electrolytes and nitrogen (calculated as crude protein) did not differ between the groups. The present findings for the effect of salt on BP do not justify the use of salt intake as a means for decreasing BP in the population.

Key words: duplicate portion technique, energy intake, intake of minerals and electrolytes, high and low blood pressure, population study, salt intake, women.

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Hypertension and overweight are important risk factors for cardiovascular disease (6, 11, 23) and there is evidence that a decrease in body weight usually leads to a decrease in blood pressure (BP) (12, 29). Therefore, weight reduction should be of value in the treatment of hypertension. However, it is common experience that weight reduction is difficult not only in the severely obese but even in the large number of women who are moderately overweight. Another

risk factor is excessive salt intake (8, 21, 31). Accordingly, a reduced consumption of salt may promote decreased BP (27) and moderate salt restriction has been used either as the sole treatment of mild hypertension (26) or in combination with diuretics (28). Owing to the results of these studies, reduced salt consumption as a dietary goal has won official approval in the USA (20).

The present investigation deals with the question whether subjects with high and low BP differ in their dietary habits, especially with respect to their intake of energy and salt. The investigation was carried out with the duplicate portion technique (3, 4).

SUBJECTS

The subjects were selected from participants in an ophthalmological survey performed in the Dalby population during 1969-70. In that study BP was measured in a strictly standardized fashion as described previously (34). The survey comprised 963 females, of whom 590 (82%) completed the study. All the latter were without antihypertensive treatment at the time of selection. From this female population, those with BP above the 95th percentile (group A, $N=36$) in the age range 40-79 years were selected and age-matched with 36 females selected from those with BP below the 30th percentile (group B). The subjects in group A were selected on the basis of systolic and diastolic BP and pulse pressure; they were included if any of these indices or any combination thereof exceeded the 95th percentile in the whole material for the five age groups 40-49, 50-59, 60-69 and 70-79 years. Subjects were assigned to group B if both the systolic and the diastolic BP were below the 30th percentile. The pulse

Abbreviations: T₄ = thyroxine, T = thyroxine, BP = blood pressure.

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pressure was not used as a selection criterion for group B in order to avoid including females in whom low pulse pressure was combined with elevated diastolic BP.

In group A one subject refused to participate while another failed to attend all investigations. A third was on antihypertensive treatment and a fourth had developed diabetes mellitus after the first examination. These four subjects and the matched controls in group B were excluded. Furthermore one female from each group failed to manage food sampling by the duplicate portion technique. Hence these two subjects and their matched controls were also excluded and each group finally comprised 10 females. In 1973 the age in group A was 57 ± 13 and in group B 59 ± 14 years (mean \pm SD).

METHODS

Anthropometric and BP measurements

Height and weight were measured according to the WHO recommendations (30). Relative body weight was calculated as weight (kg)/height (cm)² $\times 100$ and the index of obesity as weight (kg)/height (m)³ (36).

The technique for BP measurement has been described in detail (33).

Biochemical analyses

The analyses of B-haematocrit, B-glucose, B-cholesterol, B-triglycerides and B-urate levels were performed by conventional techniques. The analyses of S-triiodothyronine (T₃) and S-thyroxine (T₄) were carried out by selective radioimmunoassays (5, 25).

Sampling of food and urine

The dietary habits were investigated during Jan–May 1975. The duplicate portion technique (3, 4) was used to study the intake of energy, fat, protein, carbohydrates and minerals. Participants were informed about the technique and the duplicate portion technique and given precise instructions for urine collections. The food sampling was carried out within a period of four weeks. It comprised six days divided into three periods of two consecutive days (Monday–Tuesday, Wednesday–Thursday and Saturday–Sunday). During the second day in each of the three periods 24-hour urine specimens were collected (referred to as 1974). The participants also made up written protocols giving not only the type of food eaten but also crude estimates of the quantities expressed in colloquial terms—for each of the sampling days in order to trace the source of unexpected values.

Analyses of nutrients and energy intake

The duplicate portions were kept in the refrigerator overnight and then brought to the laboratory. After weighing the portion was homogenized, homogenized and fat-extracted with chloroform as described earlier (3, 4). In the present study, however, sodium deoxycholate as emulsifier was changed to Triton X-100 (methyl-phenoxypolyethoxy-ethanol, Kabo-Gräve, Sweden) in order to avoid sodium contamination. Total fat content was obtained after evaporation of the solvent extract. The

protein content was determined by the method of Kjeldahl (37) and the ash content was determined by the method of Kjeldahl (37). The crude protein intake was obtained by the method of Kjeldahl (37) and the crude fat intake was obtained by the method of Kjeldahl (37). The crude protein intake was obtained by the method of Kjeldahl (37) and the crude fat intake was obtained by the method of Kjeldahl (37).

Minerals (calium, magnesium, sodium, potassium, phosphorus, sulphur, chlorine, bromine, iodine, zinc, copper, manganese, iron, cobalt, nickel, selenium, molybdenum, vanadium, chromium, barium, strontium, yttrium, lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, hafnium, tantalum, niobium, molybdenum, technetium, ruthenium, rhodium, palladium, silver, cadmium, indium, tin, antimony, tellurium, selenium, arsenic, germanium, gallium, indium, thallium, lead, bismuth, polonium, astatine, radon, francium, actinium, thorium, protactinium, uranium, neptunium, plutonium, americium, curium, berkelium, californium, einsteinium, fermium, mendelevium, nobelium, lawrencium, rutherfordium, dubnium, seaborgium, bohrium, hassium, meitnerium, darmstadtium, roentgenium, copernicium, nihonium, flerovium, livermorium, tennessine, oganesson).

Furthermore the 24-hour urinary excretion of sodium, potassium, magnesium, calcium, phosphorus, sulphur, chlorine, bromine, iodine, zinc, copper, manganese, iron, cobalt, nickel, selenium, molybdenum, vanadium, chromium, barium, strontium, yttrium, lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, hafnium, tantalum, niobium, molybdenum, technetium, ruthenium, rhodium, palladium, silver, cadmium, indium, tin, antimony, tellurium, selenium, arsenic, germanium, gallium, indium, thallium, lead, bismuth, polonium, astatine, radon, francium, actinium, thorium, protactinium, uranium, neptunium, plutonium, americium, curium, berkelium, californium, einsteinium, fermium, mendelevium, nobelium, lawrencium, rutherfordium, dubnium, seaborgium, bohrium, hassium, meitnerium, darmstadtium, roentgenium, copernicium, nihonium, flerovium, livermorium, tennessine, oganesson).

Statistical methods

Mean values, standard deviation, coefficient of variation and correlation coefficient were calculated by conventional methods. Most calculations were performed by the Computer Centre, University of Lund. Statistical analyses were also made with the aid of the 101 Statistical Programme was generated and Student's *t*-test on paired or unpaired data. The variance for excretion was determined by the variance for sodium and potassium excretion on occasions (one 1973, one 1974 and one 1975). Significance levels are expressed as $P < 0.05$, $P < 0.01$, $P < 0.001$. Symbols are used: \bar{x} = mean, s = standard deviation, DF = degree of freedom, P = statistically significant ($P < 0.05$), $P < 0.01$, $P < 0.001$. All values are given as \pm SD unless stated.

RESULTS

Anthropometric measurements

The results in 1973 and 1974 are shown in Table 1. The mean values of weight, relative weight and the index of obesity were all higher in group A than in group B. The weight difference in height. All these values were essentially unchanged between 1973 and 1974.

Blood pressure

The BPs in groups A and B at the first and the follow-up examinations are shown in Table 2. The differences in systolic and diastolic blood pressure between the two groups persisted from 1973 to 1975. In 1973 the mean systolic BP was 130/80 mmHg and in 1975 130/80 mmHg.

I Anthropometric measurements in 1972

	Group A	Group B	p
Weight (kg)	161±6	163±6	n.s.
Height (cm)	161±15.7	159.7±10.1	n.s.
Body weight (kg)	124±0.27	0.96±0.17	***
Body fat (%)	18.4±4.0	13.9±2.6	***
Weight (kg)	160±6	162±6	n.s.
Height (cm)	160.4±16.1	160.5±9.8	***
Body weight (kg)	125±0.28	0.97±0.17	***
Body fat (%)	18.6±4.1	14.1±2.6	***

Initial recordings in both groups ($p < 0.01$). In Group A systolic BP had decreased ($p < 0.01$) to the initial level while in Group B it remained higher ($p < 0.01$). Diastolic BP varied between the different occasions. It was somewhat lower in Group A in 1975 than in 1969–70 and higher in Group B in 1972 than in 1969–70 (Table II).

II Laboratory variables

Hematocrit, fasting triglyceride, T_4 and S-uric acid were significantly higher in Group A than in Group B. There was no difference between the two groups in fasting glucose, fasting cholesterol or $S-T_3$ levels. The results in 1975 are given in Table III. The differences between the groups were similar in 1972 although the absolute levels were not

III Energy, fat, protein, carbohydrates and electrolytes

The intake of energy yielding nutrients—fat, protein and carbohydrates—is given in Table IV both

IV Blood pressures (mmHg) at the first study and the follow-up examinations

	Group A	Group B
BP 1972	175±34	114±8
BP 1975	190±34**	130±15***
BP 1970	179±32	123±19**
BP 1970	102±13	73±6
BP 1975	103±14	76±7**
BP 1970	97±13*	73±7

* $p < 0.01$ ** $p < 0.001$

Table III Biochemical measurements in 1975 for the two groups

	Group A	Group B	p
FS Cholesterol (mmol/l)	5.9±1.1	5.5±1.0	n.s.
FB Glucose (mmol/l)	4.8±0.4	4.6±0.5	n.s.
B Hematocrit (%)	39±2	37±3	**
FS Triglycerides (mmol/l)	1.10±0.67	0.77±0.59	*
S-Uric acid (μmol/l)	257±51	228±56	*
S-T ₃ (ng/ml)	1.79±0.27	1.72±0.20	n.s.
S-T ₄ (ng/ml)	93±19	84±12	*

in absolute amounts and in per cent of total energy. The mean values of energy and energy yielding nutrients in Groups A and B did not differ between the six sampling days and no significant differences between the groups were found for any of the studied sources of energy (Table IV). Both groups showed an average daily intake of energy around 6.3–6.4 MJ. In Group A 35% of the energy originated from fat and 38% in Group B. Protein constituted 13–14% of the energy in both groups and carbohydrates around 50%.

Neither did the intakes of minerals (calcium, magnesium, zinc, copper) and electrolytes (sodium, potassium) differ between the groups (Table IV). The sodium intake in both groups amounted to around 100 mmol daily, corresponding to an intake of salt of about 6 g/day.

Urinary excretion of minerals, electrolytes and nitrogen

The mean values for urinary excretion of minerals (calcium, magnesium, zinc, copper), electrolytes (sodium, potassium) and nitrogen (protein) showed

Table IV Energy intake during six sampling days

	Group A	Group B
Energy intake		
MJ	6.4±1.5	6.3±1.5
kcal	1525±349	1512±353
Fat (g)	58±16	62±18
	(35%)	(38%)
Protein (g)	52±15	48±11
	(14%)	(13%)
Carbohydrates (g)	189±42	180±45
	(51%)	(49%)
Sodium (mmol)	105±31	94±25
Potassium (mmol)	55±14	52±12
Calcium (mg)	577±287	577±224
Magnesium (mg)	270±85	243±66
Zinc (mg)	8.1±2.7	8.1±2.9
Copper (mg)	1.50±0.68	1.45±0.49

Table V Urinary excretion of minerals and protein (nitrogen $\times 6.25$) in studies performed in 1972 and on two occasions in 1975

	Group A	Group B
1972		
Sodium (mmol)	133 \pm 43	120 \pm 38
Potassium (mmol)	67 \pm 24	56 \pm 18
Protein (g)		56 \pm 17
1975 1		
Sodium (mmol)	146 \pm 54	125 \pm 54
Potassium (mmol)	60 \pm 19	59 \pm 19
1975 2		
Sodium (mmol)	120 \pm 32	118 \pm 28
Potassium (mmol)	59 \pm 12	59 \pm 14
Calcium (mg)	146 \pm 46	130 \pm 61
Magnesium (mg)	67 \pm 21	59 \pm 25
Zinc (mg)	0.35 \pm 0.13	0.27 \pm 0.11
Copper (mg)	0.05 \pm 0.016	0.05 \pm 0.013
Protein (g)	58 \pm 13	55 \pm 12

no differences between groups A and B (Table V). The values for sodium and potassium were virtually unchanged in both groups between 1972 and the two examinations in 1975 (Table V). Analysis of variance did not show any significant differences between the separate measurements of 24-hour urinary excretions of sodium and potassium in groups A and B respectively (Group A *F* ranged between 1.41 and 2.40 *DF*=(4 154) group B *F* ranged between 0.17 and 0.30 *DF*=(4 161)). The mean urinary excretion of protein in group B in 1975 2 did not differ significantly from the value in 1972 (Table V). The mean values of sodium, potassium and protein in the food were lower ($p < 0.001$) than the corresponding values for urinary excretion. The mean values of urinary excretion of calcium, magnesium, zinc and copper were lower ($p < 0.001$) than the consumption.

Correlations between some of the investigated parameters

The index of obesity was correlated to mean arterial BP in group A ($p < 0.001$) but not in group B (Table VI). The index of obesity showed negative correlations with the intake of fat, protein and carbohydrates in group A ($p < 0.01$) while corresponding correlations in group B were not significant (Table VI). Dietary protein, sodium and potassium were correlated to the corresponding urinary excretion values in groups A and B ($p < 0.001$ or $p < 0.01$).

(Table VI). The intakes of sodium and potassium showed negative correlations with mean BP in group A ($p < 0.001$ and $p < 0.01$) while the corresponding correlations in group B were not significant (Table VI).

DISCUSSION

Epidemiological investigations indicate that body weight or other expressions of obesity may play a strong association with hypertension. In the present study the index of obesity was significantly ($p < 0.001$) to mean arterial BP in group A (high BP) but not in group B (low BP). It is underlined that group A was recruited from females with the highest BP values among the females not on treatment for hypertension. They do not represent a group with established hypertension. But they may represent females in the early phase of primary hypertension where the changes of parameters associated with developing hypertension ought to be greater.

The index of obesity was higher in group A than in group B and correlated to a mean difference in body weight of 16 kg between the groups. The higher index of obesity could not be explained by any difference in energy intake or the intake of separate food components like fat, protein or carbohydrate. In fact there were significant negative correlations ($p < 0.01$) between the index of obesity and

Table VI Correlation coefficients between the investigated parameters

	Group A	Group B
Index of obesity mean		
arterial BP	0.65 *	0.12
Index of obesity intake of fat	-0.49 *	0.08
Index of obesity intake of protein	-0.43 *	0.04
Index of obesity intake of carbohydrates	-0.40 *	0.03
Intake of protein urinary		
excretion of protein	0.49 *	0.12
Intake of sodium urinary		
excretion of sodium	0.41 *	0.08
Intake of potassium urinary		
excretion of potassium	0.64 *	0.12
Intake of sodium mean		
arterial BP	-0.48 *	0.03
Intake of potassium mean		
arterial BP	-0.41 *	0.03

$p < 0.01$ * $p < 0.001$

fat, protein and carbohydrate in group A and group B.

The difference in body weight between the two groups may have to be traced far back in time as the index of obesity was unchanged in both groups when examined in 1972 and 1975. The diets were studied in 1975. The present finding that obese and normal weight women consumed the same amounts of energy is also in accordance with reports (9) Keen et al (16) in a recent study found no intake in relation to adiposity and did not find a highly significant inverse correlation between food energy intake and adiposity in normal British populations. It was suggested from findings that higher degrees of adiposity are associated with food energy intakes lower than normal and hence lower total energy expenditures. These cross sectional data do not exclude the possibility of a relationship between development of overweight and a concomitant progression towards higher values.

The aetiology of obesity is however a field for further study. One aspect discussed is the regulation of the metabolic rate by thyroid hormones. This is remembered when a decrease in body weight is used as a tool in the treatment of high BP. However, the consumption of energy may differ between those with a high and those with a low index of obesity. It is of interest in this context that the $S-T_4$ level was significantly higher in group A than in group B (Table III).

Another nutrient suggested to be associated with hypertension was consumed in the same amount, about 6 g, in both groups. There was no difference between salt intake and mean arterial pressure which is in accordance with previous findings in a group in elderly with a similar mean daily salt intake (4-6 g) using the duplicate portion sampling technique (4). The urinary excretion of salt was reproducible as demonstrated by analysis of the excretions of sodium and potassium of the same magnitude in the two groups and related to the intake but exceeded this by about 10% (0.001).

Only Bing et al (2) described 24-hour urinary excretion of sodium as a means of monitoring salt intake in patients with essential hypertension. The relevance of this report for the present study lies in the value for urinary excretion which was 16 mmol/24 hours. For age and sex matched with the corresponding value was 152 mmol/24

hours. The finding that hypertensive patients and normotensive subjects do not differ in the 24-hour urinary excretion of sodium is also consistent with other studies (7, 14, 22, 32). However, conflicting results have been reported on the relationship between BP and urinary sodium excretion (1, 15, 35). Thus Berglund et al (1) found that up to a resting diastolic BP of 90 mmHg sodium excretion rose in agreement with the theory of pressure diuresis (11). Above 90 mmHg, however, sodium excretion fell with increasing BP. It was suggested that the data indicated a curvilinear relationship between BP and salt intake. We actually found a very strong negative correlation between intake of sodium and BP in the group with high BP. This may be interpreted as an expression of such a curvilinear relationship even if the narrow BP distribution in the low BP group may have masked a correlation between BP and salt intake.

Before drawing further conclusions from this dietary investigation the validity of the duplicate portion sampling technique has to be discussed. The validity may be evaluated by comparing the intake with the output of sodium, potassium and nitrogen. Their magnitudes ought to be the same if the subject is in metabolic balance and the intake and excretion have been monitored under satisfactory conditions which are not yet clearly established. Sodium, potassium and nitrogen were excreted in amounts exceeding the intake, suggesting an underestimation of the food intake. There is also a technical explanation to consider. The same chemical methods of analysis were applied to very different raw materials, e.g. urine and mixed lyophilized and fat extracted food homogenate powder. In fact, losses of about 10% of sodium and potassium have been found during treatment of the food materials (4). Another possibility is unsatisfactory conditions, e.g. duration, choice of day, and number of days etc. for comparison of intake and excretion.

All current methods for measuring dietary intakes have shortcomings in reflecting a true picture of the food intake, but when two groups are compared these factors presumably affect both groups equally. Therefore it seems justifiable to conclude from the present data that dietary factors could not be implicated as a primary cause of discrepancies in BP or body weight between the two groups. This conclusion does not rule out the possibility that a diet therapy which reduces the body weight of an

obese patient with hypertension would not be successful. It must be mentioned that Parrys et al (28) observed a slight decrease in BP when the intake of salt was halved in patients with hypertension.

Roughly speaking both urinary and dietary measurements showed that the salt intake of these women with low and high BP respectively did not exceed 10 g/day. Salt restriction has been employed efficiently as evidenced by the rice fruit diet of Kempner which contained approximately 200–500 mg of sodium per day (17). When salt restriction is used to reduce BP the critical level for sodium intake seems to be very low—about 2–3 g/day as shown by hemodynamic investigations (10–19). Therefore the dietary goals outlined for the US (20) with a recommended daily salt intake of about 8 g seem doubtful. The present data regarding salt intake do not support restriction of salt intake as a tool for decreasing BP.

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Table 1 Laboratory data on the patients

Case no	Sex	Age (y)	S T ₄ (nmol/l)	Free T ₄ index	S T ₃ (nmol/l)	TSH (mU/l)	
						Before thyroxine	After thyroxine
Group 2							
1	♀	41	177	203	3.8		
2	♀	42	190	218	5.6		
3	♀	27	216	237	6.6		
4	♀	68	200	202	5.6		
5	♀	36	191	207	2.4		
Group 3							
1	♀	65	8			47	-
2	♀	49	12			40	3.4
3	♀	52	9			46	3.2
4	♂	49	7			49	3.3
Normal values			60-170	45-160	1.4-3.2	<7.2	

SUBJECTS AND METHODS

Group 1 (control subjects infused with verapamil) Five healthy non-obese volunteers 4 women and 1 man 26-56 years of age (mean \pm S.E.M. 36 ± 6 years) without family history of hypercalcaemia or diabetes.

Group 2 (thyrotoxic patients infused with verapamil) Five non-obese women with thyrotoxicosis but without family history of diabetes or hypercalcaemia 27-68 years of age (43 \pm 7). The diagnosis was based on (a) symptoms and signs typical of the disease and (b) laboratory tests outlined in Table 1. At the time of the first investigation none of the patients was on any medication. After antithyroid treatment symptoms and signs characterizing the disease disappeared gradually and all patients became euthyroid. At that time 4 of them were reinvestigated.

Group 3 (hypothyroid patients infused with verapamil) Four patients with primary hypothyroidism 3 women and 1 man 49-65 years of age (54 \pm 4) without family history of diabetes or hypercalcaemia. Features on which the diagnosis was based were (a) symptoms and signs typical of the disease and (b) laboratory data given in Table 1. When first investigated none of these patients was on any medication. After oral thyroxine treatment symptoms and signs characterizing the disease disappeared gradually in all. Three patients were reinvestigated when euthyroid.

The participating subjects were informed about the purpose of the study and gave their free consent.

Analytic procedures Blood glucose was determined enzymatically with a commercial glucose oxidase preparation (Kabi Reagents, Stockholm, Sweden). Immunoreactive insulin in serum was assayed by a two-antibody procedure essentially as described by Soeldner and Sline (24). Serum calcium was measured by a flame photometric method.

Experimental design All experiments were performed in the morning after an overnight fast and after an equilibration period of approximately 30 min. Blood samples for glucose, insulin and calcium determinations were drawn

from an antecubital vein. Verapamil was infused via the antecubital vein of the opposite side.

Group 1 Basal values for glucose, insulin and calcium were obtained at -10 and -2 min. A 10 min 25% infusion (Isoptin® Kabi, Ludwigshafen, Germany) was started and kept going at 180 min. Verapamil 15 mg dissolved in 10 ml water was infused in each experiment. Blood glucose, insulin and calcium were drawn every 10 min during verapamil infusion.

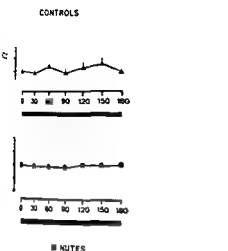
Group 2 All thyrotoxic patients were treated with verapamil as described above for 3-10 months of oral antithyroid treatment. 4 of them were investigated according to an identical experimental protocol. At that time all of them were euthyroid.

Group 3 The 4 patients with primary hypothyroidism were initially infused with verapamil in the same way as patients in group 1. When 3-10 months of oral thyroxine substitution had been instituted they were reinfused with an identical amount of verapamil during a period of 180 min.

Calculations When evaluating the effect of verapamil on the peripheral blood glucose concentration, the following calculations were made. In each individual calculation the mean of 2 peripheral glucose values before verapamil infusion were calculated. Then the individual glucose values during verapamil infusion were calculated. It was investigated whether these glucose changes differed significantly from the control level. For this purpose a *t*-test was applied on paired data. The effect of verapamil on the peripheral blood calcium concentration was tested in a similar way.

RESULTS

Group 1 In this group the mean \pm S.E.M. glucose concentration was 4.2 ± 0.4 mmol/l.



Change in insulin and glucose concentrations in response to i.v. infusion of verapamil (■) in healthy subjects (mean \pm SEM)

pooling concentration of insulin was 6.2 ± 0.6 mU/l and of calcium 2.3 ± 0.06 mmol/l. Verapamil did not affect the peripheral venous glucose or insulin concentrations (■) shown in Fig. 1.

The untreated thyrotoxic patients had a control glucose concentration of 4.7 ± 0.4 mmol/l. Control insulin and calcium concentrations were 1.9 ± 0.1 mU/l and 2.3 ± 0.04 mmol/l respectively. When verapamil was infused, glucose started to fall and reached a level significantly below control value from 60 min on (Fig. 2 left). Insulin concentration however remained unaffected by verapamil. When 4 of the 5 patients in this group

were reinvestigated at a time when their thyroid function had normalized their control glucose inulin and calcium concentrations were 4.2 ± 0.3 mmol/l, 12.0 ± 0.8 mU/l and 2.3 ± 0.04 mmol/l respectively. Verapamil did not affect significantly either the glucose or the insulin concentration at this occasion (Fig. 2 right).

Group 3 The control glucose concentration in the hypothyroid group was 4.8 ± 0.3 mmol/l before any medication was instituted. The corresponding insulin and calcium concentrations were 6.1 ± 0.4 mU/l and 2.4 ± 0.07 mmol/l respectively. Although the blood glucose concentration dropped slightly toward the end of the verapamil infusion, this fall was not statistically significant (Fig. 3 left). In contrast, the verapamil infusion elicited a transient insulin increase (Fig. 3 left). Thirty min after the start of the infusion, the peripheral venous insulin concentration had increased by $93 \pm 14\%$ ($p < 0.01$). Thereafter it dropped rapidly and did not differ significantly from the control level from 60 min on. When 3 of the 4 hypothyroid patients were reinvestigated after thyroxine substitution, their mean basal concentration of glucose was 4.7 ± 0.1 mmol/l, of insulin 8.0 ± 3.2 mU/l and of calcium 2.4 ± 0.07 mmol/l. This time however verapamil did not affect either the glucose or the insulin concentration (Fig. 3 right).

DISCUSSION

In the current as well as in a previous investigation (21) verapamil infused into healthy subjects was

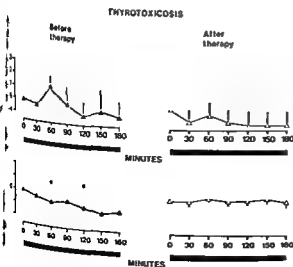


Fig. 2 Change in insulin and glucose concentrations in response to i.v. infusion of verapamil (■) in thyrotoxic patients before and after antithyroid treatment (mean \pm SEM). * $p < 0.05$, * $p < 0.01$.

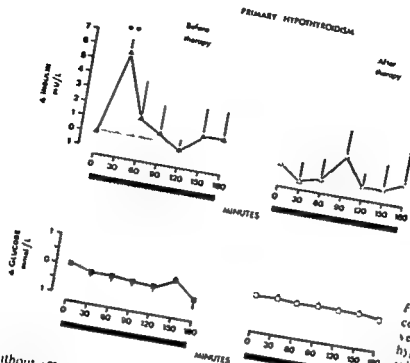


Fig. 3 Change in plasma and free concentrations in response to verapamil (■) in patients with primary hypothyroidism before and after substitution (mean \pm SEM). $p < 0.01$

without effect on both insulin and glucose concentrations (Fig. 1). In thyrotoxic patients verapamil brought about a significant fall in glucose concentration whereas insulin concentration remained unaffected (Fig. 2 left). Since verapamil thus induced a decline in glucose in the face of an unchanged insulin concentration it is reasonable to assume that verapamil might have influenced other glucose regulatory factors than insulin. Glucagon is such a factor. In dogs and in rats the pancreatic secretion of glucagon is inhibited by verapamil (10, 14). If this is also the case in thyrotoxic but not in healthy subjects the discrepancy between the glucose responses to verapamil infusion in thyrotoxic and healthy individuals seems logical. Although the α -cell secretion has been shown to be normal in thyrotoxic patients (22, 23) it is not known whether pancreatic α -cells react normally in thyrotoxic subjects when exposed to a calcium blocking agent. As long as this has not been convincingly shown the above mentioned hypothesis can neither be supported nor rejected.

Other factors involved in the glucose metabolism which may be affected by verapamil in thyrotoxic patients are catecholamines. It has been maintained that the thyroid hormones potentiate the metabolic effects of catecholamines (13). Adrenal catecholamines accelerate the release of non-esterified fatty acid (NEFA) from adipose tissue (4).

An increased plasma concentration of NE has been found in patients with thyrotoxicosis (25). Thyrotoxic patients also have an increased take of glucose thought to be related to increased availability for oxidation of NEFA, so it cannot be excluded that verapamil, by increasing the secretion of adrenal catecholamines, the turnover rate of NEFA and thereby about an increased peripheral glucose consumption, blockades of acetylcholine evoked release of catecholamines by D-600, a calcium channel blocking verapamil derivative (19), support the verapamil induced fall in glucose in thyrotoxic patients. It is interesting to note that this fall is abolished by oral antithyroid treatment restoring the thyroid function (Fig. 3 right).

Hypothyroid patients (Fig. 3 right) respond to verapamil in a different manner. The fall in glucose concentration is followed by a slight and never reached level. If changes in catecholamine concentrations suggest that insulin sensitivity has to be

pancreas has to traverse the liver before the periphery. A verapamil induced decrease in the fractional hepatic retention of insulin might be excluded in the hypothyroid patients as the insulin peak in the peripheral venous blood of these patients might have been due to a decreased hepatic retention of insulin rather than to an increased β -cell secretion. Although the current study does not provide evidence either to support or reject this possibility it shows that verapamil substitution abolishes the insulin peak and leaves unaffected insulin and glucose concentration response to verapamil infusion in hypothyroid patients (Fig 3 right). In conclusion the present investigation has shown that in abnormal and different insulin-glucose response pattern to verapamil infusion in hyperthyroid patients which can be restored to normal by medical treatment. This may have clinical significance since hyperthyroid patients often have arrhythmias and angina pectoris and thus are subjected to verapamil treatment.

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Renal Function and Morphology in Long-Term Lithium and Combined Lithium-Neuroleptic Treatment

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ACT Ten patients on long term lithium and ten on lithium and neuroleptics (combination therapy) were examined with renal biopsy and renal function. Patients on combination had more pronounced histopathological and lower concentrating capacity than patients on lithium alone. Patients on combination had received a larger total dose of lithium and had higher maximum serum lithium levels than patients on lithium alone. Patients with large renal volumes had a low concentrating capacity. There was a negative correlation between degree of pathological lesions and urinary concentrating capacity. Estimation of urinary concentrating capacity seems to be of value for the assessment of renal function in lithium treatment.

Key words: lithium, neuroleptic therapy, urinary concentrating capacity, antidiuretic hormone, kidney pathology. *Acta Med Scand* 208: 381, 1980.

It has been known for many years that lithium can cause renal lesions in animals (9). Lithium intoxication is also known to be a serious condition in man, with renal lesions following lithium intoxication (7). Reports on the renal morphology in man have been few (6, 8). The study of Hesibach et al. (8) on chronic renal lesions in patients with focal fibrosis and nephron atrophy in patients who had been on lithium treatment for several years prompted us to perform a study on the renal morphology in patients on long term lithium treatment. We found that most patients had an impaired urinary concentrating capacity even after withdrawal of lithium (4). We also found that patients who were treated with both neuroleptic drugs and lithium had a lower concentrating capacity than patients treated with lithium alone (2). The aim of the present study was to examine the renal morphology in lithium treatment and compare

it with the results of renal function tests. We also wanted to know if patients treated with both lithium and neuroleptic drugs have a renal morphology which differs from that of patients treated with lithium alone.

PATIENTS

Twenty patients on long term lithium treatment were studied. They were divided into two groups: one consisting of five males and five females, aged 52.8 ± 10.0 years, who had been treated with lithium alone (lithium group) and the other of four males and six females, aged 39.6 ± 10.2 years, who had received both lithium and neuroleptic drugs (combination group). The latter patients had been treated with so-called major tranquilizers (haloperidol, chlorpromazine, fluphenazine, perphenazine and others) in different combinations and doses for more than one year. Patients receiving only small doses of neuroleptics, e.g. for sleep disturbances, were not included. Data concerning the lithium treatment are given in Table I.

The patients had no known disorders except mental illness or side effects of drug therapy. Nine patients in the lithium group had discontinued their lithium intake two months before the examination. In the combination group, three patients had stopped taking lithium, one patient had had lithium intoxication two years before the examination and one had previously unknown hypothyroidism without clinical signs at examination. One patient in each group received thyroxine therapy for hypothyroidism.

METHODS

All patients were examined at the Unit of Nephrology, Department of Internal Medicine, Umeå University. From the patient journals we calculated how many months the patients had received lithium therapy and the total dose of lithium consumed (Table I). We also extracted from the journals all previous determinations of serum lithium concentration, which had been performed every third month or more often, in blood samples taken 12 hours after the last intake of lithium. The highest single value was noted (Table II). The blood pressure was recorded at presentation. Urinary protein and glucose were determined qualitatively. Urinary sediment was examined with phase contrast microscopy (10). The daily urine volume, serum

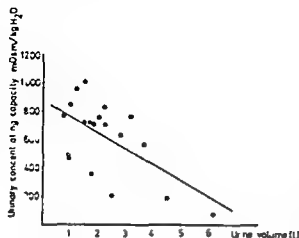


Fig. 3 Relation between urinary concentrating capacity (DDAVP test) and daily urine volume ($r=0.61$). Symbols as in Table I.

(3/10). The three patients in group C were all receiving combination therapy. There was a negative correlation between daily urine volume and concentrating capacity (Fig. 3).

DISCUSSION

The patients in this study were selected from a larger patient population on long-term lithium therapy. They were not randomly selected so this study cannot answer the question about the frequency of renal lesions in patients on long-term lithium therapy. Patients on combination treatment had more pronounced lesions in the biopsies than patients treated with lithium alone. The difference was, however, not statistically significant, which might be due to the rather crude method of grading the lesions found in the biopsies. The lesions as previously reported (4) seem to be of a nature similar to that described by Hestbech et al. (8). One explanation of the less severe renal lesions found by us than by Hestbech et al. might be that most of their patients had had lithium intoxication. The reason for our finding of more severe histopathological changes in the combination group than in the lithium group is obscure. Neuroleptic drugs per se or in combination with lithium may be harmful to the kidney. The higher maximum serum lithium concentration in the combination group might also be the critical factor. In our opinion a high peak serum lithium level is a more likely reason for the differences between the groups than the larger total

dose of lithium or the longer duration of treatment in the combination group. This is supported by the finding that the patients with the most pronounced lesions had the highest peak serum lithium level but they had also received very large total doses of lithium, longer than the others (Table II). Our results do not permit us to conclude which factor is the most important, namely of serum lithium or neuroleptic treatment. In the patients on combination therapy, the maximum serum lithium level was higher than in lithium alone. The explanation is that several patients on combination therapy were severely psychotic and had not been able to discontinue their lithium therapy adequately because of psychosis.

The renal function correlated with most of the biopsy findings. All patients with glomerular lesions had a urinary concentrating capacity less than 400 mOsm/kg, patients with tubular lesions (group C, Table II) had lower concentrating capacity. Determination of urinary concentrating capacity might be of value for the patients with renal lesions who might be able to continue the lithium therapy if the concentrating capacity is low. The indication for discontinuation of treatment should be very strict if the concentrating capacity is very low. Urinary concentrating capacity cannot be withdrawn if the patient is already controlled with frequent measurements of daily urine volume in combination with conclusions as measurement of the concentrating capacity.

ACKNOWLEDGMENTS

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PRELIMINARY REPORT

Hypoglycemia in Alzheimer's Disease

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PATIENTS AND METHODS

Four groups of patients were investigated. Group 1 120 patients (mean age 70 y) who fulfilled the criteria of dementia of Alzheimer type (insidious onset loss of memory intellectual and personality deterioration typical non-fluctuating progressive downhill course absence of hypertension stroke or other diseases causing dementia). Group 2 247 patients (mean age 71 y) with distal gangrene. Group 3 62 patients (mean age 63 y) with cerebrovascular disease. Group 4 272 nondemented nondiabetic control patients (mean age 69 y) most of whom were hospitalized because of deep venous thrombosis or heart failure.

Oral glucose tolerance tests were performed according to a standardized procedure and the areas under the glucose tolerance test curves were calculated as described earlier (2).

RESULTS AND DISCUSSION

After excluding all cases with open diabetes we found significantly lower fasting blood sugar determined by the glucose oxidation method in group 1 (4.34 mmol/l) than in all other groups: controls ($p < 0.02$), group 2 ($p < 0.005$) and group 3 ($p < 0.005$). The numerical values of the areas under the glucose tolerance test curves (2) were also significantly smaller in the dementia group than in the other three groups (Fig. 1).

The investigation suggests that patients with dementia of Alzheimer type have a changed carbohydrate metabolism with decreased blood glucose levels. In order to check whether these results are due to an underlying malabsorption or not we have undertaken a preliminary study on 9 patients with dementia of Alzheimer type and found normal values in their xylose absorption tests. Seven out of 9 patients had normal results in the vitamin A ab-

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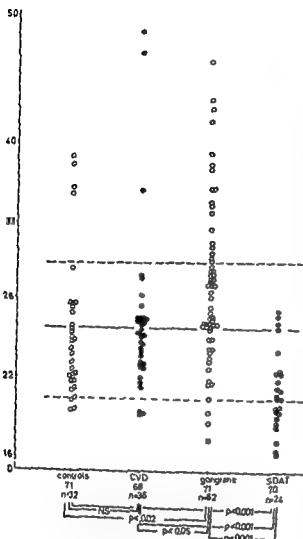


Fig 1 Oral glucose tolerance test CVD = cerebrovascular disease SDAT = dementia of Alzheimer type

sorption test the two exceptions having a slightly diminished vitamin A absorption. From these results it would appear that malabsorption is unlikely.

It seems more likely that the changed carbohydrate metabolism is due to malfunction in the hormonal regulation for example in the brain pituitary-adrenal axis in which the cholinergic and

dopaminergic transmitter systems play a tant role. Dementia of Alzheimer type suggested to be a generalized disease not confined to the brain tissue (5). Catecholamines somewhere else in the body e.g. in the liver make it difficult to mobilize the carbohydrate stores. An interesting question is to what extent at all dementia of Alzheimer type and diabetes mellitus in old age coexist. It has been suggested that for the peripheral nervous system a slight decrease in glucose levels leads to exhaustion of the neuronal firing. It remains to be found out whether or not the decreased glucose levels can impair the acetylcholine release as extensively as found by independent groups in the brain of patients with dementia of Alzheimer type.

ACKNOWLEDGEMENTS

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Effect of Different Kinds of Fibre

on Postprandial Blood Glucose in Insulin Dependent Diabetics

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Abstract Dietary fibre may retard glucose absorption in normal and diabetic subjects. It is, however, unclear which type of fibre would be most effective for this purpose. We therefore studied the effect of pectin (pectin) differs from fibre from barley (85%) and citrus (15%) (Dumovital®) in its effect on postprandial blood glucose responses. Eight insulin dependent diabetics fasted overnight and were then given a meal without their morning insulin. The basic meal was composed of 90 g white bread and 120 g jam (carbohydrate 105 g) was given three times: with 15 g pectin and with 15 g Dumovital. Blood glucose was measured for three hours. It was shown that pectin administration considerably reduced the postprandial rise in blood glucose. Dumovital showed no such effect. Barley/citrus mixture of cellulose, hemicellulose, lignin (pectin) has not the same inhibiting effect on the postprandial rise in blood glucose as pure pectin in insulin dependent diabetics. Thus, the specific type of fibre must be considered when prescribing dietary fibre to diabetics.

and dietary fibre pectin bran insulin-dependent
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pancreal aim in the treatment of diabetes mellitus is to normalize the blood glucose. Even with the use of insulin and diabetic agents and insulin, the diet is a cornerstone in the treatment. In recent decades a variety of dietary regimes have been used, but to the diabetic patient it is advised to eat a normal diet with some restrictions (1). Surprisingly little is known, however, about how different foodstuffs and mixtures of foods affect the postprandial rise in blood glucose. One of the food components which has attracted increased attention is dietary fibre. Lusk et al (5) showed that addition of pectin to guar gum (both potent gelling agents) to a test

meal diminished the postprandial rise in blood glucose and insulin in non-insulin dependent diabetics. The same was shown in insulin dependent diabetics and healthy volunteers (3-6). Guar gum and pectin given alone have the same effects. When 7 diabetics (6 on insulin, 1 on diet alone) were given 25 g guar gum daily in addition to their usual diet for one week periods, the mean urinary glucose excretion decreased 40-50% (7). The insulin dose was not reduced during the study. Other studies where diets rich in fibre (mixture of cellulose, hemicellulose, lignin and pectin) have been given to diabetics for a longer period (2 weeks-15 months) have resulted in improved diabetes control (2, 9, 10).

The above mentioned studies indicate that some types of dietary fibres are able to improve diabetes control, most likely by inhibiting the absorption rate of monosaccharides in the upper gastrointestinal tract. The effect of pectin and guar gum seems obvious, but what about other types of commercial fibre mixtures? This was our departure for comparing the effect of pectin and barley bran on the postprandial rise in blood glucose in insulin dependent diabetics.

PATIENTS AND METHODS

The study is based on experiments with eight insulin dependent diabetics who were receiving insulin twice daily. Table I contains relevant patient data. Each patient was given three different test meals (Table II): a basic breakfast alone and the same breakfast with 15 g pectin added to one component (the jam) or with 15 g Dumovital® fibre (Dumex). Dumovital is a commercial fibre mixture widely used as a dietary supplement in Norway. It is a mixture of cellulose, hemicellulose, lignin and pectin composed of 85% barley bran and 15% citrus fibre.

The test meals were given after an overnight fast. The

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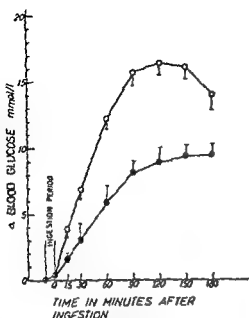


Fig 1 Average postprandial increase in blood glucose after the basic meal (O) and after the basic meal supplemented with 15 g pectin (●)

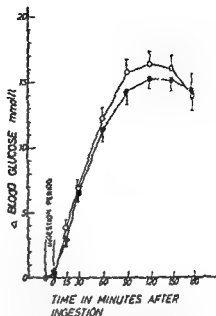


Fig 2 Average postprandial increase in blood glucose after the basic meal (O) and after the basic meal supplemented with 15 g barley bran (●)

subjects had taken their afternoon dose of insulin on the day before but did not take their ordinary morning insulin before the tests. Meals were consumed in 10 min. Capillary blood samples were taken in the fasting state and at 0, 15, 30, 60, 90, 120, 150 and 180 min following the meal. Blood glucose was determined enzymatically with glucose oxidase (Glox).

Statistics: Wilcoxon test for pair differences.

RESULTS

Figs 1 and 2 show the average increase in blood glucose (\pm S.E.M.) after each of the three meals. Addition of 15 g pectin to the basic meal gave a marked reduction in the postprandial rise in blood glucose. The difference is statistically significant at 15 ($p < 0.005$), 30 ($p < 0.025$), 60 ($p < 0.01$), 90

120, 150 ($p < 0.005$) and 180 ($p < 0.025$) min. A similar reduction was found after addition of 15 g barley bran.

DISCUSSION

Our finding that addition of pectin to a test meal diminishes the postprandial rise in blood glucose in insulin dependent diabetics is in good accord with findings by others (3, 5, 6). Addition of bran had no such effect in our patients. This can be compared with a study by Jenkins *et al.* who found that 42 g wheat bran (12 g dietary fibre) added to an oral glucose tolerance test in 61 volunteers had no significant effect on the

Table 1 Data on the patients

	Mean	Range
Age (y)	21	17-31
Duration of diabetes (y)	6	3-11
Insulin requirement (IU)		
Morning		
NPH insulin	31	24-48
Rapid acting	12	2-44
Afternoon		
NPH insulin	22	12-48
Rapid acting	6	4-10

Table 2 Composition of the test meals

Foodstuff	Weight (g)	Carbohydrate (g)	Energy (kJ)
1 Basic meal			
White bread	90	48	1040
Strawberry jam	120	56	910
Total	210	104	1950
2 Basic meal + 15 g pectin (suspended in the jam)			
3 Basic meal + 15 g Dumevital® fibre (suspended in the jam)			

rise in blood glucose and insulin. In that the large intestine transit time was measured with types of fibre. Pectin delayed the transit by 15 min, guar gum by 75 min, while bran by 45 min. These differences were based on the basis of differences in viscosity seems to be positively correlated to the effect on the postprandial rise in blood

from studies on breath hydrogen (6) excretion patterns of xylose (8) and (4) indicates that the effect of pectin is due to slower absorption of carbohydrates rather than malabsorption. Pectin and gel-forming agents could elicit various effects on the gastrointestinal tract. I. Slower gastric emptying. II. Prolonged intestinal transit time. (4) II. of carbohydrates in a gel or viscous solution could retard the diffusion of digestive products towards the absorptive mucosal surface. III. Modulation of gastrointestinal hormone responses. IV. Interference with glucose absorption at the intestinal membrane. Dumas et al. (1978) found that cellulose, hemicellulose, lignin and guar did not seem to have any effect on the absorption of carbohydrates in our short term study. But as previously mentioned, fibre mixtures may have effects on diabetes regulation in long term studies (2, 9, 10). These studies are how more difficult to interpret because in making a fibre diet, other components (e.g. the amount of carbohydrate, protein and fat) have been varied. Even though we could have short and long term effects of fibre mixtures on carbohydrate metabolism. Possible mechanisms for this could be: I. Degree of malabsorption. II. Modification of gastrointestinal hormone responses. III. Influence on the transport across the absorptive membrane.

This report clearly shows that different types of fibre can have different effects on the absorption of nutrients from the gastrointestinal tract. The specific type of fibre must be considered when prescribing dietary fibre to diabetics.

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Circulating Protein Complexes in D-Penicillamine Therapy of Rheumatoid Arthritis

Correlation between IgG and α_1 Antitrypsin IgA Complexes and Clinical Response

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ABSTRACT Eighteen of 24 patients with rheumatoid arthritis showed intermediate IgG complexes on preparative ultracentrifugation. Treatment with D-penicillamine. Serial analysis of these showed strikingly close correlation between reduction of IgG as well as α_1 antitrypsin IgA complexes. Three patients did not respond to treatment and retained both types of complexes. These virtually disappeared in all responders. Small changes were found in total IgG or IgM and the reduction of IgA levels was moderate. For one case who developed IgA deficiency importance of further analysis of non responders noted.

Key words: penicillamine treatment rheumatoid arthritis protein complexes. *Acta Med Scand* 208 393 1980.

As being a metal chelator D-penicillamine has a strong affinity for thiol groups in vivo forming mixed sulphides with extracellular thiols like cysteine and some plasma proteins. Normal human plasma contains S-S linked complexes between α_1 antitrypsin and IgA which are cleaved on mild reduction in vitro (8). The level of the α_1 antitrypsin IgA complexes decrease rapidly in rheumatoid arthritis (RA) patients receiving D-PA treatment (15). Circulating IgG complexes considered to contain IgG RF are frequently demonstrated in patients with RA (10, 13). Also these complexes decrease after D-PA therapy (11).

This paper is an extension of these observations with the aim to study the behaviour of both common and simultaneously in the course of D-PA treatment in RA.

MATERIAL AND METHODS

Clinical material

The effect of D-PA treatment on circulating IgG complexes was studied in RA patients at the Department of Rheumatology County Hospital Örebro.

Sera were drawn from all patients before therapy and then at control visits at irregular intervals. All sera were kept at -20°C until analysis. Sera from 24 out of 66 patients were subjected to gradient centrifugation analysis. Before D-PA treatment sera from 17 of these 24 patients contained circulating IgG complexes; mostly of intermediate size. These sera and subsequent sera from the same patients were analyzed for the presence of α_1 antitrypsin IgA complexes and IgG complexes. Moreover the serum values of IgG and IgA and IgM were determined as well as titres of rheumatoid factor (RF).

All patients from whom sera were taken fulfilled the American Rheumatism Association criteria for definite or classic RA. The initial D-PA dose was 250 mg/day and was increased monthly to 750 mg in a few patients to 1000 mg.

Gradient centrifugation analysis

This was performed in a Spinco SW 50 rotor as described earlier (10). The fractions obtained after gradient centrifugation were submitted to radial immunodiffusion assay for IgG. If IgG was detected in fractions corresponding to sedimentation coefficients >9S this indicated presence of IgG complexes.

Determination of IgM, IgG and IgA

Immunoglobulins were determined by single radial immunodiffusion (9).

α_1 Antitrypsin IgA complexes

These were demonstrated by the crossed immunoelectrophoresis technique (2). The complex concentration was estimated from the area under the precipitation arch in the β zone when using rabbit anti- α_1 antitrypsin in the gel of second dimensions on run (8, 15).

Abbreviations: D-PA=D-penicillamine RA=rheumatoid arthritis RF=rheumatoid factor

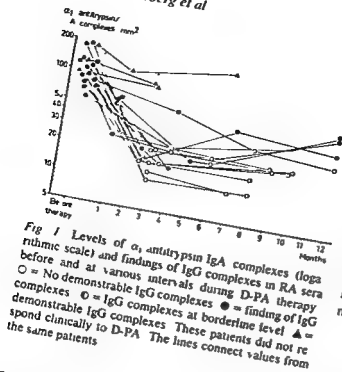


Fig 1 Levels of α_1 antitrypsin IgA complexes (logarithmic scale) and findings of IgG complexes in RA sera before and at various intervals during D-PA therapy. \circ = No demonstrable IgG complexes \bullet = finding of IgG demonstrable IgG complexes Δ = finding of IgG respond clinically to D-PA. The lines connect values from the same patients.

Determination of α_1 -antitrypsin in serum
This was performed by the electroimmunoassay according to Laurell (7)

Determination of rheumatoid factor in serum
The Latex fixation test with human gammaglobulin was performed with reagents from Hyland Laboratories Los Angeles California

RESULTS

Figs 1 and 2 show determinations of α_1 antitrypsin IgA complexes and the findings of IgG complexes in sera from 17 RA patients before and at various intervals during (Fig 1) and after D-PA therapy (Fig 2). The pretreatment levels of α_1 antitrypsin IgA complexes were within the expected ranges. As can be seen from Fig 1 the values decreased after D-PA therapy and remained at low levels during treatment. The behaviour of the IgG complexes paralleled closely that of the α_1 antitrypsin IgA complexes. In samples taken 3 months after initiation of therapy IgG complexes could be demonstrated only in single sera. In 8 patients D-PA had to be withdrawn depending on various side-effects. In all sera taken 1-12 months after therapy the α_1 antitrypsin IgA complexes had reappeared and reached approximately the initial levels. IgG complexes were demonstrated in all but one sample (Fig 2).

Three patients did not respond to D-PA. IgG com-

plexes were demonstrated in all samples during treatment and the levels of α_1 antitrypsin IgA complexes were higher than in the responders. As can be seen from Fig 3 the IgG IgA values decreased slightly during treatment. Differences between values before and after D-PA therapy were statistically significant ($P < 0.01$) tested by χ^2 test. In one patient with noglobulin values within the normal range, D-PA therapy the initial IgA value 18 g/l decreased to 0.45 g/l after one month of treatment. After three months it was 0.1 g/l and remained that level after withdrawal of more than 12 months D-PA therapy. The RF titres decreased more than IgM values (Fig 4).

DISCUSSION

The capability of sulph hydriol reagents to dissociate 19S RF and macroglobulins prompted Jaffe to try D-PA treatment in RA. It was however soon realized that direct action of RF was not a likely mode of action for D-PA (5). A decrease in IgM RF titre that was demonstrated in most cases is obviously not due to dissociation of the IgM molecule. The usually more pronounced in IgM RF titre of the whole IgM serum pool. Moreover the IgM RF titres usually persist for months after initiation of D-PA treatment which is difficult to reconcile with a direct reducing effect on the molecule. Pritchard and Vukobratovic (12) present a hypothesis postulating a normalizing effect of D-PA on pathologic structures of IgG in RA.

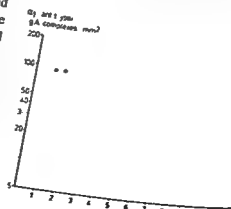


Fig 2 Levels of α_1 antitrypsin-IgA complexes and findings of IgG complexes in RA sera at various intervals during D-PA therapy. Symbols as in Fig 1.

lead to less RF formation. The production of IgM RF but only small IgM may be interpreted to support which however needs further direct proof.

characterized by a strong tendency to disulphides *in vivo*. It is excreted in the form of D-PA cysteine disulphide. A possible explanation of the disappearance of IgA complexes would be the blocking of the penultimate SH group on newly formed IgA (15). This would lead to a slow fall in treatment and a slow increase after D-PA administration. The IgG complexes are immune complexes containing antibodies to various antigens. They are not known to involve intermolecular disulphide bonds. The behaviour however of IgG complexes in the D-PA treated RA was strikingly similar as in their reduction by reducing agents *in vitro*. This may indicate a similar mode of action of D-PA on both IgG and IgA complexes. It is of special interest that the complex persistence previously observed in non-responders (14, 15) was again confirmed in this series.

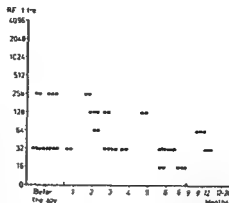


Fig. 4. Latex titres in RA sera before and during D-PA therapy.

We have evidence that non-responders did absorb and metabolize D-PA. The reduction in their plasma cysteine levels and urinary excretion of D-PA cysteine mixed disulphide did not differ from that of the responders (14, 15). One must therefore look for other explanations of their unresponsiveness.

Laurell (personal communication) obtained recently unexpected findings when administering radiolabeled α_1 -antitrypsin complexed with likewise labeled cysteine kappa chains and IgA respectively. The disappearance rates of the complex components were more or less identical with those of the free components. This strongly indicated that the complexes are in rapid exchange rather than long-lived. The SH blocking theory for D-PA action is not supported by these experiments.

Altered redox equilibrium leading to a relative depletion of cysteine and a reduced intracellular redox potential may be the relevant biochemical action of D-PA in RA. Rapid elimination of D-PA and its metabolites (4) calls for explanation of the rather gradual return of the protein complexes after withdrawal of D-PA. It is conceivable, although speculative, that D-PA may give rise to secondary enzyme induction involving redox regulation which may affect physiologic and pathologic complexes some time after treatment. Along the same line, non-responders might show some aberrant regulation of their redox balance, making them resistant to intracellular reduction by D-PA. Further analyses of primary non-responders as well as patients in therapeutic escape would be of great interest.

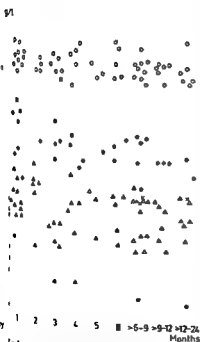


Fig. 5. IgG and α_1 antitrypsin values (log) in RA sera before and at various intervals after D-PA therapy.

ACKNOWLEDGEMENTS

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Flexible Fiberoptic Bronchoscopy in Sarcoidosis

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Twenty nine patients with clinical X ray and histology consistent with sarcoidosis have been studied with flexible fiberoptic bronchoscopy (FFB) and scalene node biopsy. The yield from bronchial mucosal biopsies was 43% from transbronchial lung biopsy. 43% of the patients showed epithelioid cell changes with FFB (lung and/or mucosal biopsies) with 79% with scalene node biopsy. Three showed extensive bronchial mucosal changes suggesting processes due to sarcoidosis. Sarcoidosis of the bronchial mucosa is a frequent finding. FFB is the best method for discovering bronchial sarcoidosis with or without stenosing bronchitis. In our opinion, FFB has a place as a method in diagnosing sarcoidosis.

bronchial mucosal biopsy flexible fiberoptic bronchoscopy lung biopsy sarcoidosis scalene node biopsy

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Several methods are available today for obtaining histological material from patients with sarcoidosis. The lymph node biopsy (2) is a well known procedure with a high diagnostic yield in 6-12%. Biopsies can also be taken from bronchial mucosa (3-11, 14) and the lung (15). Using flexible fiberoptic bronchoscopy (FFB) as a method which has been much discussed in the literature (16-18).

The aim of this study was to compare the diagnostic yield from FFB with a well known diagnostic method, the scalene node biopsy, and to find out if FFB has any place in the routine investigation of patients with suspected sarcoidosis.

PATIENTS AND METHODS

In an eight month period (Feb.-Sept. 1977) we studied 29 new cases of sarcoidosis seen at our department. There were 14 women and 15 men with a mean age of 48 (range 22-70). The age and sex distribution in our study is shown in Table I. The patients were assigned to one of

three stages according to their chest X ray findings: stage I (hilar lymphadenopathy), stage II (pulmonary infiltration) and stage III (pulmonary fibrosis).

FFB was performed under topical anaesthesia. Bronchial mucosal biopsies were taken from three standard places: 1) right upper lobe bronchus, 2) right middle lobe bronchus, 3) left upper lobe bronchus, and elsewhere when macroscopical changes in the mucosa were seen. Transbronchial lung biopsy (TBL) (1) was taken from the right lower lobe in 14 of the 29 patients. TBL was performed only in patients in stages I and II; one to three biopsies were taken from each patient. Scalene node biopsy was performed in 28 of the 29 patients.

If neither scalene node biopsy nor bronchoscopy gave histological evidence of sarcoidosis, other methods such as biopsies from skin, muscle, and mediastinoscopy were applied for achieving histological evidence of sarcoidosis.

RESULTS

Of the 29 patients, 13 had a chest X ray consistent with stage I, 13 with stage II and 3 with stage III. In 28 of the 29 patients studied, scalene node biopsy and/or biopsy with FFB revealed non-caseating epithelioid cell granulomas. The diagnostic yield from each method is shown in Table I. The histological evidence of sarcoidosis was obtained by mediastinoscopy in one patient. Of 28 patients with positive biopsies from scalene node biopsy and/or FFB, 9 had positive biopsies with both methods, 6 with FFB alone and 13 with scalene node biopsy alone.

There was no significant difference in the diagnostic yield from any of the three standard places of bronchial mucosal biopsy (Table II). Biopsies taken from microscopically changed areas, however, showed much higher frequency of sarcoid infiltration. TBL was performed in only 14 patients and lung tissue was obtained from all of them. The result is shown in Table I.

Abbreviations: FFB = flexible fiberoptic bronchoscopy, TBL = transbronchial lung biopsy.

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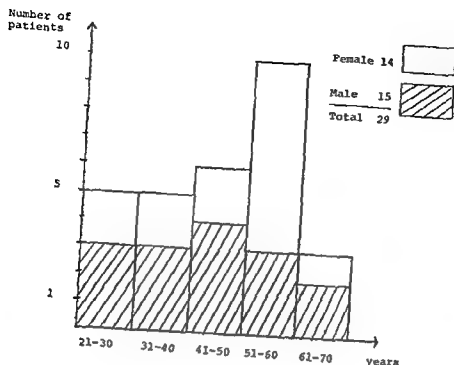


Fig 1 Age and sex distribution of 29 patients with sarcoidosis

During bronchoscopy we found macroscopical changes in the bronchial mucosa in 15 (50%) of our patients. Thickened inflamed bronchial mucosa was found in 13 patients and was the most usual macroscopical change. Yellow white plaques were also often seen in the larger bronchi of some patients. The mucosal thickening was extensive in three patients leading to almost total bronchial stenosis. Two patients had stenosis of the right middle and upper lobe bronchi both with partial atelectasis on the chest X ray. Stenosis of the apical segmental bronchus of the left lower lobe was seen in one patient.

DISCUSSION

Non caseating epithelioid cell granuloma with sarcoidosis were found in 4 patients with a higher frequency in stage I (31%). This result is in line with studies by others (11-14). Ståhle (11) found bronchial lesions as fine parallel runs like a broom, mucosal swelling, focal stenosis of the bronchi and flat yellow plaques. In our study 50% of the patients had macroscopical changes of the same type. The frequency of sarcoid infiltration like in our study (10/14) was found in biopsies taken from macroscopically changed areas.

We found a low diagnostic yield with TBL. This method revealed sarcoid granulomas in 6 (six out of 14 patients). The reason for the low frequency of positive biopsies with TBL was probably the low number of biopsies taken from each patient. In other studies (4-5) with six to eight biopsies from each patient the diagnostic yield with TBL was 83-90%. The diagnostic yield with TBL thus increase by taking multiple biopsies (11).

The total diagnostic yield with FFB was 79%. If tissue and mucosal biopsies were taken the diagnostic yield would be much higher than that found by Teister (13). The difference is probably due to the low diagnostic yield from TBL in our study (6/14) and the high diagnostic yield from FFB (22/28) and 79% positive scalene

Table 1 Diagnostic yield from FFB and scalene node biopsy in 29 patients with sarcoidosis (no of patients with positive biopsies/total of patients biopsied)

Stage	FFB		TBL and/or mucosal biopsy	Scalene lymph node biopsy
	Mucosal biopsy	TBL		
I	4/13	3/6	6/13	9/12
II	6/13	3/8	7/13	10/13
III	2/3	0/0	2/3	3/3
Total	12/29	6/14	15/29	22/28
%	41	43	52	79

Result of mucosal biopsies from different locations in the bronchial tree (no of positive biopsies taken)

Bronchial mucosal biopsies

Right upper lobe	Right middle lobe	Left upper lobe	Macroscopical changes elsewhere in the bronchial mucosa
1/13	2/13	0/13	1/4
3/13	4/13	4/13	3/6
1/3	2/3	2/3	0/0
5/29	8/29	6/29	4/10
17	28	21	40

According to the literature (6, 7, 10, 12) comparing the diagnostic yield from scalene node biopsy and FFB in the present study the latter seems preferable. Lung tissue or mucosal biopsy were however positive in patients in whom Daniel's biopsy failed to give a correct diagnosis of sarcoidosis.

An interesting feature in our study was the finding of extensive changes in the bronchial mucosa in some patients leading to a partial bronchostenosis. An X-ray will in such cases often show a narrowing and may sometimes arouse suspicions of a neoplasm (9). These patients have often no symptoms with cough, wheezing and so on, and the prognosis seems rather poor (3). Early corticosteroid treatment could perhaps prevent chronic fibrotic bronchostenosis.

In our opinion, in spite of the rather low diagnostic yield in the present study, a place as a diagnostic method in the diagnosis of sarcoidosis. Mucosal biopsy is a necessary procedure for establishing the diagnosis of bronchial sarcoidosis, especially in cases of bronchial stenosis. The combination of mucosal biopsy and bronchoscopy will in most cases give histological evidence of non-caseating granulomas in patients with sar-

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Epidemiological Studies in the Upernavik District, Greenland

Incidence of Some Chronic Diseases 1950-1974

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An epidemiological survey of several in the Upernavik district, Northwest reported. The study population (ap- inhabitants) is one of the remaining whal populations in Greenland. It was the 25 year period 1950-74 as to the he diseases, which was based on all cases in hospital during this period. The disease the Greenlanders differs from that of an populations, having a higher fre- quency and epilepsy but a lower frequen- of acute myocardial infarction, diabetes thyrotoxicosis, bronchial asthma, multiple psoriasis. The distribution of cancer is from that of the Danish population, but incidence of cancer is of the same mag- comparable studies should be per- Greenlandic districts that are character- profound changes in life style, in order e the effect of these changes on the disease

incidence chronic diseases Greenland
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the population of Greenland mainly Es been living under extreme conditions for of years epidemiological studies there to elucidate etiological factors of diseases. (1) showed in a comprehensive study pattern of diseases in the population of differed considerably from that in Den applies to chronic as well as acute dis

ertelsen's survey the disease pattern in has not been investigated systematically ch studies would be of special interest

in view of the intense industrialization and the changes in standard of living which have taken place in Greenland during the last 20-30 years

We have therefore carried out an epidemiological study with regard to fertility, mortality and the incidence of some chronic diseases observed during 25 years in a population of whalers and sealers in Greenland. The present report deals with the disease pattern; the results concerning fertility and mortality will be presented elsewhere.

MATERIALS AND METHODS

Study population

The study covers the Greenlandic population, i.e. persons born in Greenland and/or with Greenlandic mothers in the district of Upernavik in Northwest Greenland demarcated by the Thule district (lat. 75° N) and the Umanak district (lat. 72° N). In 1950 the district had a population of Greenlandic origin of 1398 persons and 1887 persons in 1974. The proportion of persons of other origin (mostly Danish) remained constant at 5-10%.

The population living in Upernavik town and ten settlements along the coast is traditionally occupied mainly with whaling and sealing, fowling and to a lesser degree fishing. This traditional diet can be supplemented by Danish food. Apart from periods of construction work (harbours, schools, etc.) the only occupation in whaling and sealing or employment in public services or administration. During 1950-74 social development was concentrated to Upernavik town where trade, schools, tele-communications and public administration underwent considerable development. In 1952 30% of the Greenlandic population of the district were living in Upernavik town; in 1972 37%.

The housing standard in the district as a whole has improved during the last 30 years, as the turf huts of earlier times have been replaced by standard wooden houses.

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Table 1 Number of observed person years distributed by sex, age and observation periods

Age (y)	Males		Females	
	1950-63	1963-74	1950-63	1963-74
0-6	2 749	3 119	2 096	1 753
7-14	1 686	2 895	1 843	2 611
15-24	1 719	1 425	1 738	1 387
25-39	1 654	1 907	1 684	1 860
40-64	1 633	1 643	1 643	1 671
≥65	194	392	784	376
Total	9 135	11 391	9 788	10 658

The hospital (established in 1950) is located in Upernavik town and employs two Danish doctors, nurses trained in Denmark and assistants trained in Greenland. The settlement of Kaulshavn in the northern part of the district, some 150 miles from Upernavik town, has a small nursing station. Apart from better facilities for admitting patients to other hospitals, primarily in Godthåb—the capital of Greenland—the health facilities have not changed much since 1950.

Study material

The study covers the 25 year period 1950-74 and the following diseases are included: cancer (all types), apoplexy, epilepsy (grand mal), peptic ulcer, acute myocardial infarction, rheumatoid fever, chronic polyarthritis, chronic glomerulonephritis, chronic glomerulonephritis, diabetes mellitus, psoriasis, psychosis (manic-depressive reaction), psychosis and schizophrenia, multiple sclerosis and thyrotoxicosis.

From the files of the Upernavik Hospital we registered all cases of the above mentioned diseases in which the final diagnosis had been made during hospitalization in the study period. The name, date of birth and date of death, when applicable, were recorded together with the exact date of diagnosis.

Statistical data

The distribution by sex and age (6 age groups) of the population of Greenland is known from censuses carried out at varying intervals as a rule about every five years. Furthermore, the numbers of males and females registered births, deaths and migrations of the Greenlandic population are known for each year. Detailed information on migrations is lacking in the local public registry but it is known that in 1960-74 there were 1540 new arrivals and 1790 departures from the district corresponding to a average annual arrival and departure rates of 3.8% and 5.7% respectively of the population. Unfortunately it is not known whether the same individuals move back and forth just as the age of the individuals moving is unregistered. It is also probable that not all migrations are registered.

Analyses

On the basis of the data on age and sex, together with those on the size of the study population, we estimated the

number of person years observed in each age group, assuming that the person years for those leaving the district cancel out. For the assessment of changes in the incidence of the diseases, we decided to subdivide the study period into 1950-63 and 1963-74. Since the age structure best estimated the standardized total number of person years during the first period, we used the person years in the age classes for the standard.

RESULTS

The material comprises 149 diagnoses of individuals 8 of whom entered the diagnoses each.

To permit further analyses, the data are presented separately in an appendix showing the number of person years in each age and sex group and observed in Table II. Surveys of the cases observed of the study period can be compared case by relating the standardized number 63 with the number observed in 63. Furthermore, we show the expected number (total study period) estimated on the European mainly Danish age specific rates.

DISCUSSION

The value of epidemiological studies which present are based on hospital records is a large measure on the validity of the data. The completeness of the material. This is a concern in the present case, as the study is widely scattered with limited possibilities of communication in certain seasons and have only limited facilities for diagnoses. However, specialized assistance obtained either from district consultants or referring the patients to Godthåb or Denmark.

The diseases mentioned are mostly easily diagnosed even on a purely clinical basis. Most likely the results contain underestimation to the absence of cases which remained unnoticed or were treated out of hospital by the district doctor visited the settlements in order to obtain a homogeneous basis for estimates that we decided to restrict ourselves to the only regional hospital. Tolerance of the sources of error will be different in relation to each following sections.

The cases distributed by diseases, sex and observation periods

of numbers with the age structure for 1963-74 as the standard are given in parentheses

	1950-62		1963-74		1950-74	Expected no 1950-74 (♂ + ♀)	Refer- ence no
	♂	♀	♂	♀	♂ + ♀		
tract incl	4 (5.0)	11 (11.1)	13	11	46	53	1
glands	0 (0.0)	4 (4.2)	4	1	9	13	3
tract	0 (0.0)	0 (0.0)	2	2	4	13.7	3
	3 (4.0)	0 (0.0)	2	4	9	5.1	3
	0 (0.0)	3 (3.1)	0	2	5	5.6	1
tal system	0 (0.0)	3 (2.9)	1	4	7	8.1	3
	1 (1.0)	1 (0.8)	3	1	6	1.5	3
unspecific	0 (0.0)	0 (0.0)	1	4	5	17.1	3
	6 (10.1)	5 (5.5)	8	6	25	15.0	6
(grand mal)	5 (5.9)	5 (7.0)	2	4	16	8.0	9
	4 (4.5)	2 (2.1)	10	3	19	29.0	2
	5 (6.0)	1 (1.1)	2	3	11		
polyarthritis	3 (3.7)	5 (5.4)	0	1	9		
	2 (1.8)	0 (0.0)	3	5	10	8.0	5
lonephritis	0 (0.0)	2 (1.9)	2	0	4		
is	0 (0.0)	1 (1.3)	0	1	2		
ycardial infarction	0 (0.0)	1 (1.0)	1	1	3	40.0	11
	0 (0.0)	1 (1.1)	1	0	2	40.0	10
	1 (1.0)	0 (0.0)	0	0	1	25.0	16
melitus	0 (0.0)	0 (0.0)	1	0	1	9.0	19
diucosis	0 (0.0)	0 (0.0)	0	0	0	7.0	12
rosis	0 (0.0)	0 (0.0)	0	0	0	2.0	8

found 46 cases of cancer (17 males and 29

The diagnosis had been confirmed by his-
tological examination and/or operation in all but a
few mostly old patients admitted in the ter-
minal phase in whom the diagnosis was based ex-
clusively on clinical findings. The incidence doub-
led in the first part of the observation period to
and was primarily due to more cases among
the increase could be explained by impro-
vements and/or increased frequency of
diagnosis. This applies especially to the male
population in which cancer is generally appearing
at older ages where diagnosis and attempts at
treatment have been stepped up.

The distribution of the various types of cancer is
shown in Table II together with the expected num-

ber on Danish incidence rates (3). The total
number of males and females is somewhat lower

We found an excess of parotid and
salivary gland cancers in males as well as females.
This confirms the findings of recent studies (13)
that the number of cases of lung and gastrointes-
tinal cancers was however

The low occurrence of lung cancer seems credible
as throughout the study period the total population
has had annual X-ray examinations in connection
with the mass tuberculosis campaign (18). Persons
suspected of cancer would thus be examined more
closely. It is remarkable that the four cases of lung
cancer occurred in the second part of the study
period possibly a consequence of the fact that
cigarette smoking became more common during
and after the Second World War.

Of the seven cases of female genital cancers two
were vaginal, one cancer of the ovaries—all diag-
nosed in the beginning of the study period—and
four cervix cancers diagnosed in 1972-74. This
finding is in accordance with the results of Nielsen
et al. (15) who described an almost explosive de-
velopment of cervix cancer in Greenland on ac-
count of increasing promiscuity.

Apoplexy, acute myocardial infarction

The material includes a total of 25 cases of apo-
plexy, equally divided between the first and second
parts of the study period. The diagnosis was purely
clinical in all cases and the result must be consid-

ered a minimum as many fatal cases are not included and less serious cases especially those from minor settlements have not been hospitalized. It is worth noting the considerable excess of this disease compared to the Danish population—only 15 cases should be expected on the basis of figures from a Danish study (6).

On the other hand acute myocardial infarction is rather rare. We found only 3 cases of which two must be considered uncertain due to incomplete data in the hospital records and unspecific ECG changes. Based upon experience from a Danish study we had expected about 40 cases (11).

The low incidence of acute myocardial infarction combined with the high incidence of apoplexy might be related to the reduced platelet aggregation ability in Eskimos (4). This might well serve as a protection against coronary thrombosis at the same time as it involves a greater bleeding tendency leading to apoplexy on a hemorrhagic basis.

Epilepsy

In this group we have included only cases of grand mal epilepsy which did not occur together with other diseases (mongolism, cerebral palsy, etc.). Because it is doubtful whether cases of psychomotor and petit mal epilepsy have been diagnosed in Greenland such types of epilepsy have not been included. The diagnosis of grand mal epilepsy is based primarily on information or observation of at least two convulsive attacks unrelated to rise of body temperature; furthermore a large proportion of the cases were verified by EEG during admissions to Danish hospitals.

The number of cases (16 altogether) is twice that expected from Danish conditions (9) which confirms previous results from Greenland (7). Surprisingly the incidence during the first part of the observation period was twice that in the second part.

Peptic ulcer

The number of cases of this disease is subject to some reservation since the food in Greenland often causes retrosternal pains and ructus. Especially at the beginning of the study period when X-ray examinations were limited and of poor quality the diagnoses were based on clinical findings only and it might be a matter of chance whether a case was diagnosed as gastritis or peptic ulcer. Furthermore it must be assumed that slight attacks of the disease never led to hospitalization. In 3 cases during the

first and 9 during the second part of the period the diagnosis was verified by one or the following findings: Large niche on X-ray, haematemesis or the finding of ulcer at operation. We found 19 cases against 25 expected from Danish experience (7). The discrepancy could be explained by undiagnosed cases, a lower frequency of admissions especially at the beginning of the observation period. On the other hand the increase in the number of cases from the first to the second part of the period may be real.

While the ratio between stomach and ulcer in the above mentioned Danish study is 1:5 we found stomach ulcer twice as duodenal ulcer.

Other diseases

These diseases are of rare occurrence and results should be treated with reservation.

The pronounced reduction in cases of *arthritis chronica* during the study period has an obvious explanation apart from a possible relation with improved housing conditions. It is noted that serious cases of this disease were admitted to hospital for examination (rheuma tests) this being a condition for an invalidity pension. Four patients from the first and one from the second period had in addition typical joint changes, rheumatic nodules, rheuma tests or effect of gold treatment.

The increase in psychoses (only subacute manic-depressive and reactive psychoses) is probably misleading as a psychiatric service has existed in Greenland only since 1960. In addition probably only a limited number of psychiatric cases are being admitted (noticed) in Greenland as a consequence of problems. We found 5 manic-depressive, 2 schizophrenias and 3 reactive psychoses. Cases were expected (5) but as the ascertainment of psychoses may be higher in Greenland.

The low occurrence of bronchial asthma may also be emphasized (1 case observed against 16 cases expected according to Danish conditions (16)). The case was a middle aged male and not been diagnosed in children.

Diabetes mellitus was found in only one who during hospitalization for hyperadipositas showed increased fasting blood

beta glucose tolerance curve. The patient died with diet. On the basis of a Norwegian (9) 9 hospitalized new diabetes cases should have died during the study period of which 5 were of the early insulin dependent type. The frequency of diabetes mellitus in Greenland has only been reported by Sagild et al. (17) and perhaps—as far as the insulin-dependent type is concerned—be related to the low frequency of Eskimos of the HLA antigens generally associated with this form of diabetes. In this connection the absence of thyrotoxicosis (expected according to a Danish investigation (12)) is striking as it may be assumed that immunoreactions play an important role in the etiology of pathogenesis of this disease. Diabetes was found in two persons—both of Danish and Greenlandic origin but not related with fair complexion. In a study from the Islands (10) the annual incidence was estimated to be one new case per 1 000 inhabitants. The number of cases thus expected in our study population would be at least 40, as younger people in the disease most often appears from a young age. Even if some cases never figure in hospital there can be no doubt as to the relative importance of the disease, especially among Greenlanders of preponderant Eskimo origin. Usually it should be emphasized that no cases of multiple sclerosis were found. The expected number according to Danish incidence rates (18) would be about 2. This disease has never been found among Greenlanders. We can conclude that the pattern of the diseases in our study differs from that of Western Europe as we have found frequent occurrence of epilepsy and grand mal epilepsy, but rare or non-existence of acute myocardial infarction, diabetes mellitus, thyrotoxicosis, bronchial asthma, multiple sclerosis and psoriasis. Furthermore, the distribution of cancer types is different, although the total incidence of cancer equals that of Western Europe. During the study period there was possibly an increasing incidence of cervix cancer and lung cancer. Epilepsy and chronic polyarthritis seem to have decreased during the period. Even though urbanization and industrialization is advanced in the few remaining sealing districts of Greenland, including our study population, the way of life has in many places become more European. Thus it is remarkable that diseases like

acute myocardial infarction and diabetes mellitus have not become more frequent.

In spite of the restrictions and reservations on account of the size of the material we present it for its descriptive value and also for purposes of comparison with studies in other districts in Greenland with more profound social changes. The study has been designed with special reference to such future investigations.

ACKNOWLEDGEMENTS

Financial support was obtained from the Danish Medical Research Council (grant no. 512 8623) and the Commission for Scientific Research in Greenland.

APPENDIX

Cases of chronic diseases in Upernavik district, Greenland, 1950 to 1974 (age at diagnosis, year of diagnosis in parentheses).

Cancer of the upper respiratory tract incl. salivary glands Males 60 (68), 47 (71), 68 (72), 44 (74) Females 55 (54), 27 (57), 33 (57), 63 (62), 60 (67) *Cancer of the lungs* Males 48 (63), 62 (64) Females 57 (66), 62 (70) *Cancer of the digestive tract* Males 59 (57), 59 (60), 66 (60), 66 (69), 74 (70) Females 39 (65), 80 (65), 65 (69), 42 (71) *Cancer of the breasts* Males 0 Females 49 (50), 49 (50), 34 (51), 45 (69), 60 (69) *Cancer of the urogenital system* Males 74 (65) Females 45 (50), 15 (59), 37 (62), 25 (72), 35 (72), 47 (73), 21 (74) *Sarcoma* Males 47 (58), 41 (64), 73 (72), 22 (73) Females 27 (57), 0 (64) *Others and unspecified* Males 70 (73) Females 0 (63), 69 (63), 111 (69), 8 (72) *Apoplexy* Males 44 (52), 70 (58), 64 (60), 65 (61), 111 (62), 70 (62), 67 (63), 62 (64), 74 (65), 74 (67), 61 (69), 86 (69), 76 (74), 79 (74) Females 56 (50), 25 (51), 64 (51), 78 (51), 43 (62), 69 (65), 57 (66), 66 (66), 66 (68), 60 (70), 75 (74) *Epilepsy (grand mal)* Males 24 (53), 14 (56), 15 (59), 3 (60), 30 (60), 27 (64), 10 (65) Females 8 (51), 14 (51), 5 (59), 7 (59), 10 (60), 1 (63), 7 (60), 4 (70), 2 (74) *Peptic ulcer* Males 25 (56), 37 (59), 50 (59), 35 (60), 24 (63), 58 (64), 44 (69), 32 (70), 41 (71), 43 (71), 56 (72), 66 (73), 35 (74), 54 (74) Females 32 (56), 49 (59), 32 (69), 39 (69), 50 (71) *Rheumatic fever* Males 33 (51), 10 (54), 31 (54), 17 (55), 29 (60), 33 (64), 12 (70) Females 30 (59), 17 (65), 12 (70), 22 (74) *Chronic polyarthritis* Males 31 (55), 111 (56), 8 (59) Females 30 (50), 26 (53), 13 (54), 49 (58), 17 (59), 24 (64) *Psychosis* Males 20 (51), 50 (59), 41 (69), 49 (70), 44 (71) Females 35 (63), 41 (64), 37 (71), 45 (71), 56 (74) *Chronic glomerulonephritis* Males 30 (68), 11 (69) Females 15 (63), 31 (58) *Chronic pyelonephritis* Males 0 Females 38 (59), 83 (73) *Acute myocardial infarction* Males 31 (73) Females 52 (59), 63 (71) *Psoriasis* Males 7 (74) Females 26 (55) *Bronchial asthma* Males 41 (60) Females 0 *Diabetes mellitus* Males 55 (71) Females 0 *Thyrotoxicosis* Males 0 Females 0 *Multiple sclerosis* Males 0 Females 0

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Aneurysm of the Popliteal Vein as a Cause of Pulmonary Embolism

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ACT A case of recurrent pulmonary embolism secondary to thrombosis in an aneurysm of vein is described. The source of embolism was not obvious clinically, and it was discovered after venography. The anomaly was treated by surgical intervention and the patient was free of further embolism. We have found this case in the literature.

pulmonary embolism popliteal vein aneurysm

Scand 208 407 1980

ords of a metropolitan county in Ohio. The total incidence of pulmonary embolism disregarding age and underlying disease is also unknown and probably not diagnosed in the majority of cases (3). One can only speculate that pulmonary embolism is underdiagnosed in apparently normal persons.

We would like to report a case of pulmonary embolism of unusual origin in a popliteal vein aneurysm in a healthy young man.

CASE REPORT

A 37-year-old man, previously healthy except for varicose veins of the legs, was admitted to the hospital after a syncopal attack at home. At arrival he complained of breath-correlated pains in the right upper thorax.

Clinical examination revealed a tachycardia of 120/min and an acute thrombophlebitis of the distal part of the greater

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Fig 1 Lung scintigram on the day of admission showing perfusion defect in the right upper lobe.



B

Fig 2 Venogram of the left leg showing venous defects and popliteal aneurysm of the popliteal vein with intraluminal filling defects and contrast in the femoral vein



Fig 3 Lung scintigram showing progression of the perfusion defects mainly on the right but also on the left side

vein of the left leg. The blood pressure was 110/70 mmHg. Physical examination of the heart and lungs as well as chest X-ray revealed normal findings. ECG on admission showed S-Q_T syndrome and a slight deviation indicating a right ventricular load. The deviation disappeared during the following days. The patient improved during the first few days, but still had moderate fever and slight chest pains at admission. Serial estimations showed slight increase in S-ASAT (0.46–1.44 μ kat/l) and S-ALAT (1.2–2.1 μ kat/l), the upper normal limit in our laboratory for both enzymes. On the fifth day there was a scintigram.

The scintigram showed a rather large perfusion defect in the ventral part of the right upper lobe (Fig. 1). A chest X-ray was still normal. Heparin and aspirin were given.

Examination of the left lower extremity revealed a sacral abscess, approximately 4.5 cm in diameter, extending from the dorsal part of the popliteal vein and masses of thrombotic material with protrusion of the thrombus into the distal part of the vein (Fig. 2).

The patient was transferred to another hospital for a new lung scintigram showing progression of the defects in both lungs (Fig. 3). A venogram of the left leg was normal. The aneurysm was resected and the vessel closed with direct over and over suture. A new venogram two days later showed complete obstruction of the vein at the site of the aneurysm.

On the fourth day a thrombus in the distal part of the vein was resected by Fogarty technique and the vessel closed with a saphenous patch graft.

The patient was discharged on oral anticoagulant seven days after the second operation. Lung scintigram after the second operation was normal. At six months follow-up he remained well, has no recurrence of pulmonary embolism and is free from his postthrombotic leg

DISCUSSION

This patient presented with acute pulmonary embolism and superficial thrombophlebitis of the left leg. However, this was not the source of embolism but a venogram revealed an aneurysm of the popliteal vein which was clinically unsuspected. A lung scintigram supported the diagnosis of pulmonary embolism.

Anomalies of the deep veins of the lower extremities are rare and usually unilateral (2) and this is to our knowledge the second case described with pulmonary embolism secondary to unsuspected aneurysm of the popliteal vein (4).

Pulmonary embolism constitutes a very important cause of morbidity and mortality and so far venography is the only single most useful investigation in diagnosing suspected deep vein thrombosis (5). This method is helpful not only in outlining the site and extent of the thrombus but also in the decision about surgical intervention. Other common tests such as fibrinogen scanning and plethysmography can only diagnose a thrombus but are of no help in accurately outlining the source of embolism if it is a vascular malformation.

In patients with migrating superficial thrombophlebitis deep vein thrombosis occurs simultaneously in about 20% (6) why we recommend a venogram in these cases.

In patients with clinical symptoms of pulmonary embolism a chest X-ray is usually normal like in

our patient and does not exclude this diagnosis. A positive perfusion lung scintigram in combination with a normal chest X-ray gives an accurate diagnosis.

The postoperative result with secondary thrombosis in the popliteal vein was identical to the case referred to (4). A temporary arteriovenous fistula distal to the venous reconstruction should probably have been employed in the first operation in order to increase flow and prevent rethrombosis. We have used that principle with encouraging results in more than 90 cases with acute iliofemoral venous thrombosis.

This case strongly stresses the importance of perfusion lung scanning in patients with clinical signs of pulmonary embolism and of venography in cases of pulmonary embolism of obscure origin. Venous reconstruction probably with a temporary arteriovenous fistula will prevent further embolism.

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Multiple Myeloma and Gastric Carcinoma

Possible Late Effects of Limited Abdominal X Irradiation

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A man, aged 34, was treated in 1954 for a duodenal ulcer by antroduodenectomy followed by X irradiation to the stomach in a dose of 2000 rads. Two decades later he developed several conditions attributable to the previous irradiation, including physical appearances of premature ageing, atrophy of the left kidney due to irradiation nephropathy, immune deficiency, multiple myeloma of the bone marrow and, lastly, carcinoma of the stomach. The effects of local X irradiation are discussed in relation to the known effects of total body irradiation causing decreased longevity in animals and cancer in man.

Multiple myeloma gastric carcinoma X irradiation
phthisis
Scand 208 411 1980

Long term deleterious effects of low doses of radiation in man are attracting considerable attention. Persuasive evidence has come from observation on the occurrence of cancer in persons given therapeutic irradiation for various benign conditions, exemplified by an excess of leukaemia and carcinoma of the lung and stomach after irradiation for ankylosing spondylitis (3) and in Japanese atomic bomb survivors exposed to nuclear bomb irradiation.

In our unit has kept under surveillance a group of patients who were given limited X irradiation during the 1940-60 to reduce gastric acidity as a therapy for peptic ulcer (23). This case report records a unit of degenerative and neoplastic conditions in an elderly aged man 10-20 years after irradiation, possibly attributable to this.

CASE REPORT

In 1954 a male patient aged 34 presented with epigastric pain due to duodenal ulcer of several years duration. After unsuccessful medical treatment he was treated surgically by antroduodenectomy followed three weeks later by X irradiation in an estimated dose of 2000 rads. The stomach was irradiated through anterior and posterior portals in the left upper quadrant of the abdomen as described by Scott et al (23). Thereafter he remained free of symptoms of duodenal ulcer.

In 1964 investigations revealed mildly impaired renal function: blood urea 7.5 mmol/l (normal laboratory range 2.5-8.3) creatinine clearance 74 ml/min (normal laboratory range 90-150). Also in 1964 an observation in the case record noted that he appeared prematurely aged in relation to his chronological age of 44 years and a peroral biopsy of the gastric remnant showed chronic atrophic gastritis marked by a heavy infiltration of lymphocytes and plasma cells and glandular atrophy. In 1972 he had evidence of benign prostatic hypertrophy for which transurethral resection of the prostate was performed and intermittent claudication in both legs after walking 200 yards. Renal function tests again showed mild impairment and an intravenous pyelogram showed functioning kidneys: the left measuring only 11 cm in length and the right 12 cm. The physical appearances of premature ageing were again noted. Immunological function tests for cell mediated immunity included delayed type hypersensitivity responses to five ubiquitous antigens and induction of sensitization to DNCB (13): these showed non reactivity.

In 1973 the patient complained of back pain. X ray examination revealed osteolytic lesions in both femora, left humerus and skull and degenerative changes in the lumbosacral spine. Serum electrophoresis revealed a low level of IgM and a paraprotein identified as being of IgA class with lambda light chains. Other investigations consistent with multiple myeloma revealed an ESR of 104 mm/hour, Bence Jones protein in the urine, a Hb level of 10.9 g/100 ml with a normochromic normocytic blood film and a bone marrow aspirate containing 29% plasma cells. Renal function had further deteriorated: the blood urea then being 13.7 mmol/l and the creatinine clearance 12 ml/min. A barium meal X ray examination suggested an abnormality at the anastomotic site but gastroscopy



Fig. 1 Physical appearance of the patient at age 47 considered to represent a prematurely aged man.

showed only mild narrowing at this site and multiple biopsies showed chronic atrophic gastritis.

The multiple myeloma was treated with cycles of cytotoxic drugs: melphalan 37 mg/day and prednisolone

10 mg/day for four days and prednisolone 20 mg for six days repeated every 3 weeks. Between 1964 and 1975 there were about 40 urinary infections.

In 1976 the patient presented with dyspepsia, epigastric pain and anorexia and aged considerably older than his 57 years. Fibrogastroduodenoscopy showed a gastric carcinoma surgically resected. Postoperatively he developed a chest pneumonia. The peritoneal specimen showed a cancer at the anastomosis. The gross specimen was a mucin-producing adenocarcinoma with atrophic gastritis in the gastric remnant.

Abnormal findings at autopsy included bronchopneumonia, a shrunken left kidney and a normal sized right kidney weighing 150 g. The sclerotic microscopically of the medium-sized arteries, arcuate arteries and the hilar arteries of the kidney with changes of benign nephrosclerosis of the right kidney and no evidence of metastatic gastric carcinoma.

DISCUSSION

The patient described here was free of a peptic ulcer by antacid adenectomy and irradiation to the left upper quadrant of the abdomen in a dose of approximately 7000 rads. The peptic ulcer by gastric irradiation was described by Ricketts et al. in 1948 (19) and was treated with surgery in this unit in 1953-54. Between 1500-7000 rads. Left-sided renal atrophy developed by Thompson et al. (25) in a long-term follow-up of the irradiated patient.

After partial gastrectomy and irradiation



Fig. 2 Gross specimen of the stomach showing multiple nodules of metastatic carcinoma.



Kidneys obtained post mortem from patient show a shrunken left kidney - presumably resulting from radiation to left kidney 22 years previously

ained well for over 10 years but then developed a sequence of conditions which might be due to antecedent irradiation. The first abnormalities noted on routine follow up were mild impairment of renal function and physical appearance of premature ageing. Two neoplastic conditions, multiple myeloma and carcinoma of the stomach, developed thereafter and death occurred from operative infection at the age of 57. Premature ageing admittedly is a wholly subjective assessment as there are no accepted quantifiable indices of ageing but was independently noted by observers. Decline in cell mediated immunity is known to occur in advanced age (13) demonstrable in our patient at the age of 52. The effect of irradiation in accelerating natural ageing has been reported in man and animals (2) and in a study on mice given varying doses of X rays found that longevity correlated with the early appearance of diseases as well as with human ageing notably with the

greying of hair and nephrosclerosis. Conard et al (6) in a study on humans described acceleration of ageing processes by characteristics assessed on physical examination (7) in a Marshall Islands population exposed to fall out from atomic bomb tests. Accelerated ageing was considered to be a late effect of radiation and a manifestation of non-repairable injury. Anderson et al (1) reported a reduction in life span in survivors of the Hiroshima and Nagasaki atomic bombs especially evident in those irradiated at an early age. Among atomic bomb survivors according to Jablon (12) premature deaths were due entirely to cancers and if such causes were excluded no non-specific life shortening effect was evident. All of these reports however related to total body irradiation and there is no information on decreased longevity attributable to local irradiation.

Gastric irradiation causing left-sided nephritis has been previously reported from this unit (25) and is further exemplified by the present case. Abnormal renal function being detected on routine follow up 10 years after irradiation and shrinkage of the left kidney being shown later by intravenous pyelography. The unilateral shrinkage of the left kidney found at autopsy together with suggestive vascular damage (20-25) indicated that radiation damage was responsible. It is recognised that unilateral radiation nephritis should not prejudice renal function but there were less advanced arteriosclerotic changes in the right kidney possibly attributable to scattering of the X-ray beam.

The effect of irradiation in inducing neoplasia is well established. Brown and Doll (3) reporting on 14000 patients given radiotherapy to the spine for ankylosing spondylitis found a marked increase in leukaemia, aplastic anaemia and cancers especially of the lung, stomach and lymphoreticular system. The highest frequency of cancer in the above study (7) was in the more heavily irradiated sites and cancer increased with increasing time from irradiation rising to twice the expected incidence 9-14 years after radiotherapy. Thyroid cancer is a well known complication of antecedent irradiation to the head and neck (24). In addition irradiation from atomic explosions in Japan is incriminated as a cause of various cancers (12-26). Although cancer of the stomach is not usually included among cancers induced by atom bomb irradiation perhaps because the prevailing high incidence of gastric carcinoma in Japan obscures such an increase.

ANNOUNCEMENTS

The Tenth Second Postgraduate Institute for Clinical and Clinical Cerebral Pathology is to be given at The Johns Hopkins University School of Medicine and the Johns Hopkins Hospital Baltimore Maryland USA March 22-April 3, 1981. The entire course is given in English. The full two-week program is designed for pathologists who are certified (or qualified) by the American Board of Pathology (ABP) or their international equivalents.

Applications to be made before Jan. 8, 1981. For details write John H. Frost MD, 610 Pathology Building, The Johns Hopkins Hospital Baltimore Maryland 21205 USA.

RIAS Adlectedure in Monoclonal Antibodies and Lysosomal Enzymes in Human Diagnosis and Monitoring of Therapy May 6-7, 1981 and *3rd International Symposium on Calciprotein Hormones Metabolism and Clinical Applications* May 8-9, 1981 will be held at Gardone Riviera Italy.

Deadline for receipt of abstracts Feb. 15, 1981.

Organizing Secretariat: Fondazione Giovanni Lorenzini Via Monte Napoleone 23, 20121 Milan Italy.

The 7th International Symposium on Systemic Infection sponsored by the Serono Symposia International will take place in Athens Greece June 1-3, 1981. The meeting will include invited lectures and a limited number of free communications. Congress language is English.

Deadline for receipt of abstracts (max. 250 words) Jan. 15, 1981.

Further information: Prof. S. Rappaport Center and Artificial Pancreas Ltd., Dept. of Clinical Therapeutics, Athens University, P.O. Box 115, Athens 606 Greece.

International Symposium on Endocrine and Reproductive Problems will be held at Athens June 10-17, 1981.

Subject of the meeting will be coordinated by the Scientific Committee. Deadline for receipt of abstracts (English) Feb. 15, 1981.

Information: Organizing Secretariat, Prof. Giovanni Lorenzini Via Monte Napoleone Milan Italy.

International Symposium on Cerebral and Systemic Disorders in Surgery and Medicine will be held at Gardone Riviera (Garda Lake) Italy June 4-6, 1981. Deadline for receipt of abstracts March 1, 1981.

Organizing Secretariat: Fondazione Giovanni Lorenzini Via Monte Napoleone 23, 20121 Milan Italy.

17th International Congress of Chemotherapy held in Florence Italy July 19-24, 1981.

Topics: Antimicrobial chemotherapy and immunotherapy as well as immunology and chemotherapy. Deadline for receipt of abstracts Feb. 15, 1981.

For information: Secretariat, 1st International Congress of Chemotherapy Via de' Sordani 1, Florence Italy.

Bacteriuria in a Population Sample of Women

Prevalence Characteristics Results of Treatment and Prognosis

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ACT The prevalence of bacteriuria was in a population sample of women aged 38-60 and ($\geq 100\,000$ bacteria/ml of the same species consecutive specimens) or probable bac ($\geq 100\,000$ in the first and 20 000-90 000 bac of the same organism in the second specimen) were found in 5% of the women, increasing 5% in women aged 38 to 8.6% in women aged 54. *Escherichia coli* predominated (90% of those with asymptomatic bacteriuria), and 91% of the *Escherichia coli* strains were sensitive to sulphonamides. About 10% of the women who were non bacteriuric after 6 months treatment had one or more recurrent urinary tract infections during a two-year follow up. Serum creatinine and urine concentrating capacity did not differentiate women with significant or probable bacteriuria from the others. Special radiological changes were found in 17% of bacteriuric women who were submitted to intravenous urography. When re-examined six years after the first study 23% of initially bacteriuric and 5% of initially non-bacteriuric women had bacteriuria. Patients with renal lesions have been followed for ten years but none of them has developed progressive renal impairment. Three women with chronic pyelonephritis and acute pyelonephritic attacks in the past were discovered when screening the concentrating capacity in this population sample but they were not bacteriuric when screening for bacteriuria. A study of this kind must be conducted over many years before valid conclusions can be drawn.

KEY WORDS: population study, women, bacteriuria, renal lesions, prognosis.

Acta Med Scand 208 417 1960

of screening for bacteriuria in non pregnant women are contradictory. Many investigators are of the view that search for asymptomatic bacteriuria is hardly of preventive value and that treatment suitable for large scale use is

ineffective (1-18). Furthermore the prognosis for non pregnant women with asymptomatic bacteriuria is generally good. However the natural course of bacteriuria in the long run is still unknown and the natural history of chronic pyelonephritis is enigmatic. There is still no certain way of detecting the minority of patients who are at risk of renal damage. Until a test to identify these patients has been developed, as wanted by O Grady (14) and others, it seems important that longitudinal studies are prolonged over many years.

We report on the prevalence of bacteriuria in a population sample of women initially studied in 1968-69. The screening was part of a comprehensive project including hematological, cardiovascular, metabolic, gynecologic and psychiatric studies. This study gives an account of the types of bacteria, symptoms, results of treatment and incidence of recurrent infections. The majority of women were re-investigated after 6 years in 1974-75 and those with repeated infections and/or renal lesions have been followed for ten years.

STUDY POPULATION

A population study of women in five age strata (38-46, 46-54 and 60 years) was carried out in Göteborg, Sweden during 1968-69 (3). Altogether 1462 women participated (90.1% of those sampled). Further details of the sampling procedure and characteristics of the non-participants have been published earlier (3). The way of sampling ensured that the women were representative of the female population of Göteborg in the ages studied.

Six years later in 1974-75 the same population sample was re-studied. On this occasion 1302 women participated which means 89% of the participants in the first study. Further details of the second study are given elsewhere (4).

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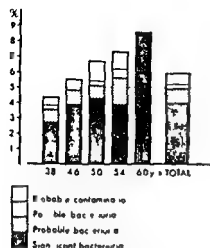


Fig. 1. Prevalence of bacteriuria in a population sample of women. The broad line denotes those who received treatment.

METHODS

In both studies the women were asked to come in the fasting state in the morning and were examined at various stations in a fixed order (3-4). They had been asked not to void urine at home if possible. Clean voided midstream urine specimens were kept in a refrigerator until cultured in the Bacteriological Laboratory within six hours. A 0.003 ml urine sample was streaked twice on a blood agar plate with a calibrated loop and 0.05 ml were spread on a plate of lactose bromthymol blue (mod Fed Disinfectant) agar (10). Counts of *Escherichia coli* and other *Enterobacteriaceae* could be estimated to a lower limit of 70/ml. When the plates were crowded the urine sample was requantitated after serial dilution. A simplified O-vero-grouping (11-13) was performed on all *E. coli* strains which were saved as deep agar stab cultures under sterile paraffin oil in the dark. Antibiotic sensitivity tests were performed by a single disc agar diffusion method (16).

Women were regarded as non-bacteriuric if the urine specimen contained 0.000 gram negative rod bacteria or 100 000 gram positive bacteria per ml. The others delivered at least one additional urine specimen 7-10 days after the first test.

Four categories of bacteriuria were defined: 1) Significant bacteriuria: two specimens each containing $\geq 100 000$ bacteria/ml of the same *E. coli* O-group or of the same species other than *E. coli* or a single specimen containing $\geq 100 000$ bacteria/ml simultaneously with symptoms of urinary tract infection requiring immediate treatment. 2) Probable bacteriuria: one specimen with $\geq 100 000$ and another with 0.000-10 000 bacteria/ml of the same organism as defined above. 3) Possible bacteriuria: one specimen with $\geq 100 000$ and another with 0.000 bacteria/ml of the same organism or ≥ 0.000 of a different organism. 4) Probable contamination: one specimen with $\geq 100 000$ and another with 100 000 bacteria/ml of gram-positive organisms belonging to the normal urethral and genital flora.

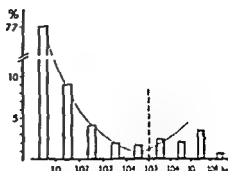


Fig. 2. Distribution of effective units in 160 women from the first 1440 women studied.

Women with significant or probable bacteriuria were treated with tablets containing 0.25 g sulphadiazine (Sulapraz) and 0.1 g sulphamethoxy pyridazine (Sulapraz) tablets bid for ten days. Women who had experienced adverse reactions from sulphadiazine 0.5 g amp 11 ml (Doklaklin®) were treated for five days followed by 0.05 g nitrofurantoin (n® Pharmacia) tid for ten days. None were then examined at scheduled regular intervals.

Statistical methods: Conventional methods for calculating mean values and standard deviation (S.D.). The hypothesis of no difference between was tested with the χ^2 test.

RESULTS

Prevalence of bacteriuria

The prevalences of all categories of bacteriuria are shown in Fig. 1. In the total sample 6 women had significant or probable bacteriuria. The prevalence of significant bacteriuria with age-specificity (Fig. 2) shows that bacteriuria had significant bacteriuria in half of women with probable bacteriuria and that contained $\geq 100 000$ bacteria/ml.

Table 1. Prevalence of bacteriuria in 160 women from the first study.

Age (y)	Total sample	Significant bacteriuria
38	32	0
46	431	0
50	338	0
54	180	0
60	61	0
Total	1462	6

ii Bacterial findings

	n	%
aerobic bacteriuria (n=58)		
<i>Escherichia coli</i>	52	90
<i>Staphylococcus aureus</i>	2	3
<i>Streptococcus</i>	1	2
<i>Staphylococcus</i> coagulase negative	2	3
<i>Neisseria meningitidis</i>	1	2
aerobic bacteriuria (n=13)		
<i>Escherichia coli</i>	12	92
<i>Staphylococcus aureus</i>	1	8
aerobic bacteriuria (n=4)		
<i>Escherichia coli</i>	4	100
contamination (n=11)		
<i>Streptococcus</i>	4	36
<i>Staphylococcus aureus</i>	1	9
<i>Staphylococcus</i> coagulase negative	1	9
<i>Bacillus</i>	1	27
<i>Neisseria meningitidis</i>	1	9
<i>Neisseria meningitidis</i>	1	9

Results

Bacterial findings are listed in Table II. *E. coli* was found in 90% of the women with significant bacteriuria and in 92% of those with probable bacteriuria. The distribution of *E. coli* per ml of urine in 1650 specimens from the first 1440 women is shown in Figure 1. Two peaks are recognized: one of non-inoculated but contaminated specimens, the other of inoculated specimens. Less than 2% of the specimens had *E. coli* counts of 10 000–100 000/ml.

Of the 68 strains of Enterobacteriaceae causing bacteriuria for which treatment was given, 91% were sensitive to sulphonamides (Table III). Three of six women with sulphonamide-resistant *E. coli* had recently been treated with sulphonamides for current urinary tract infections.

Results of treatment

Long-term follow-up. We treated 67 of 71 women in the way described. Three of them were given sulphonamides in spite of their sulphonamide-resistant bacteriuria. Four women were treated elsewhere. At one week after treatment on urine samples from 65 of these 67 women revealed that seven (11 of those checked) were still bacteriuric, six containing *E. coli* and one enterococci. One *E. coli* strain was sensitive to sulphonamides and five were resistant, one already before treatment. Two of three sulphonamide-resistant strains had disappeared after 12 days of sulphonamide treatment.

Table III Sensitivity of gram-negative bacilli to sulphonamides in women with significant or probable bacteriuria

	n	%
<i>E. coli</i>	56/64	91
<i>Klebsiella</i>	2/2	100
<i>Proteus</i>	2/2	100

Long-term follow-up. In addition to the examination about one week after treatment, the women were checked up after 3, 6, 9, 12, 18 and 24 months. The cumulative frequency of recurrent infection is shown in Figure 3. Four women who did not attend all follow-up examinations and those seven who had infected urine shortly after treatment are not included in the figure. This means that 56 women who were non-bacteriuric after treatment were followed up for two years or until infection reappeared. About half of these women (52%) became bacteriuric ($\geq 100 000$ bacteria/ml) on at least one occasion during the period. Including those with bacteriuria shortly after treatment and two women who were not checked up until about six months after treatment, 38 (58%) of 65 women initially treated and followed up had persistent bacteriuria or recurrent infection.

Side effects of antibacterial treatment. Three (5%) of 65 women infected with sulphonamide-sensitive bacteria reported previous side effects of treatment with sulphonamides. Four (6%) reported abdominal pain, dysuria or discomfort during sulphonamide treatment. These symptoms were however hardly due to the sulphonamide treatment. None of eight women treated with ampicillin and nitrofurantoin reported side-effects.

Table IV Urine concentrating capacity (<700 mOsm/kg H₂O in women with bacteriuria (significant or probable) compared to the total population sample of women

Age (y)	Women with bacteriuria		Total population sample of women	
	n	%	n	%
38	2/11	18	48/353	14
46	6/19	32	68/411	17
50	5/20	25	101/386	26
54	3/9	33	93/178	30
60	5/5	100	34/77	44

Table V Abnormal radiological findings in the urinary tract of women with significant or probable bacteriuria

Patient no.	Age (yr)	Radiological findings	Bacteria of initial infection	Bacteria of recurrence
1	38	Renal hypoplasia and/or chronic pyelonephritis	E. coli	Enterococcus
2	38	Renal calcification	E. coli	
3	38	Renal stone	Proteus	Proteus
4	46	Vesico-ureteric reflux	Proteus	Proteus and E. coli
5	46	Renal hypoplasia	E. coli	E. coli
6	50	Renal papillary necrosis	E. coli	E. coli
7	50	Renal scar (vascular?)	Staphylococcus coagulase negative	Klebsiella
8	54	Hydroureter	E. coli	E. coli

Six year re-examination A urine specimen was again examined in 1974-75. Out of the 67 women initially treated for bacteriuria 57 (85%) participated in the re-examination. Thirteen (23%) had significant bacteriuria compared to 63 (87%) of those 1236 who participated in the follow up study and who had initially not had significant or probable bacteriuria. The difference was statistically significant ($p < 0.001$).

Characteristics of women with significant or probable bacteriuria

Symptoms of urinary tract infection Seven (10%) of 71 women with bacteriuria defined as significant or probable reported dysuria. Another four reported back pain located to the kidney region in two. If the latter complaints are regarded as symptoms of urinary tract infection 11 women (15%) reported such symptoms.

Renal function All women with significant or probable bacteriuria had serum creatinine values below the upper limit of the normal range ($\leq 106 \text{ mol/l} = 1.2 \text{ mg/100 ml}$).

A urine concentration test (osmolality during the

13th hour of thirst) (2) was performed in 1974 in women with significant or "probable" bacteriuria. A maximum osmolality of $< 600 \text{ mOsm/kg H}_2\text{O}$ was considered significantly decreased and had at their examination. Of the 64 women participating in the test two had concentrations below this level. One of these two women had a normal concentrating capacity after administration of pitressin ($> 600 \text{ mOsm/kg H}_2\text{O}$) while the other had a decreased concentrating capacity also after pitressin and could thus be considered to have impaired renal function.

Twenty-one of the bacteriuric women had an osmolality below $700 \text{ mOsm/kg H}_2\text{O}$ during a 13-hour of thirst (Table IV). When compared with urine osmolality below this level the concentrating capacity tended to be lower in women with bacteriuria in some age strata. This difference was not significant.

Results from excretory pyelography During the follow up period 46 of the 67 women were subjected to excretory pyelography. Mainly those without evidence of bacteriuria refused. Pathological changes were found in 8 (17%) of the 46 women (Table V).

Leucocyte counts in the peripheral blood ESR did not differ between women with significant or probable bacteriuria and other women in the population sample (Table VI).

Women with recurrent bacteriuria

Women in whom bacterial infections recurred after treatment were offered further treatment as outpatients at the Department of Nephrology. Of these 23 women had reduced concentrating capacity ($< 700 \text{ mOsm/kg H}_2\text{O}$ after pitressin) and five had borderline values ($700-800 \text{ mOsm/kg}$).

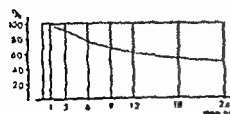


Fig. 3. Long term follow up of 46 women treated for bacteriuria and free from bacteria after treatment. Hatched area denotes recurrences, white area no recurrences.

1. Leucocyte counts in peripheral blood and ESR in women with bacteriuria (significant and compared to the total population sample of women)

Women with bacteriuria			Total population sample		
n	Mean	S D	n	Mean	S D
n (10 ¹¹)					
13	1.7	1.6	363	5.6	1.8
1	6.0	2.0	419	5.5	1.7
0	5.5	2.1	395	5.3	1.6
10	5.0	1.0	180	5.1	1.6
7	5.2	1.5	81	5.2	1.7
mmh					
13	11.3	9.6	372	10.9	11.0
1	10.7	6.7	431	11.0	8.4
0	14.5	11.0	398	12.0	8.4
10	13.1	8.9	180	15.5	15.0
7	11.4	12.1	81	17.7	13.6

no further cases of concentrating defect led to those found at the population screen

patients one of whom had normal concentrating capacity had borderline values for serum creatinine 106–120 $\mu\text{mol/l}$ or 1.2–1.4 mg/100 ml. The patient had a unilateral small kidney and radiological picture and laboratory findings of congenital hypoplasia.

Of these 24 women had pathological radiological findings (Table V) unilaterally reduced size and blunted calyces in three one of had also vesico ureteric reflux. Micturition cystography had not been performed in the others. One patient had bilateral papillary necrosis. In three patients one kidney had double calyces and ureters. No calyceal changes but a kidney probably vascular was found in one patient. Of the patients with reduced concentrating capacity had a normal urogram.

One patient developed hypertension which required treatment. Borderline blood pressures (diastolic 90–105 mmHg) were noted in two patients. In all patients had further recurrences of bacteriuria. In a few cases combined with symptoms of pyelitis. Antibacterial treatment was given repeatedly. Except for women with normal renal function and normal radiological findings the patients have been followed for up to ten years. No patient has demonstrated progressive renal impairment during this period. The patient with renal papillary necrosis has developed increasing cavities in the kidney. She is suspected for analgesic abuse.

DISCUSSION

An increasing prevalence of bacteriuria with increasing age and a marked predominance of *E. coli* with a large majority of the strains being sensitive to sulphonamides was found in this study in agreement with results from previous studies of women (7–9, 17).

The method of collecting and culturing urine specimens seemed to be very reliable, at least as far as *E. coli* is concerned. The distribution of bacterial counts of *E. coli* was similar to that achieved when catheterized specimens were used (8).

As in previous studies of bacteriuria the majority of bacteriuric women did not report any symptoms. There were no differences in leucocyte counts or ESR between bacteriuric and non bacteriuric women indicating that the majority of women had no serious infection. Similarly impaired renal function measured as serum creatinine or urine concentration capacity was not overrepresented in this group of women.

At the 6-year follow up the prevalence of bacteriuria in the total sample of women was unchanged 5% whereas the corresponding percent age of women treated for initial bacteriuria was 23. Women with abnormal radiological findings seemed to be especially prone to new infections which has also been found in previous studies (16). Pathological radiological findings were detected in 8 women. One woman had decreased concentration capacity although the intravenous pyelography was normal.

It is interesting to compare these results with the outcome of the concentration screening performed

in the total population sample in 1968-69 (2). Eleven women had a urine osmolality even after a pitressin tannate test, and renal disease was found in ten of them. Two of them are identical with patients in the present study. Three of the patients discovered by the concentration screening had a past history of acute pyelonephritis, but were non bacteriuric at the time of the initial population study.

Thus individuals with renal lesions, probably due to earlier urinary tract infections, may be missed in a population screening of bacteriuria. However, a general recommendation of simultaneous screening of bacteriuria and concentrating capacity seems not to be realistic. A simple screening test for concentrating capacity, e.g. overnight thirst, gives many false positive results and must be followed by a more reliable test, like the pitressin tannate test. On the other hand, several cases of early renal lesions are bound to be overseen by screening procedures.

One aim of a cross sectional population study is to find out how many of the bacteriuric individuals have pre-existing renal lesions. Here a clinical concentration test can be used to select patients for a pyelography.

Another and certainly an important aim is to establish a base for longitudinal studies of the consequences of bacteriuria. It is of interest to define the consequences in patients with as well as without initial lesions. A clinical long term study in Göteborg has demonstrated that frequent recurrences of urinary tract infections accelerate the progress of renal insufficiency in chronic pyelonephritis (5). The majority of patients with clinical and radiological picture of chronic pyelonephritis and with renal function reduced to about 50% often demonstrated a very slow progress over a decade.

There is no reason to believe that the progress could be faster before the function is reduced to 50%. Thus five years is a short period in a population study, and even a 10-year period is certainly not long enough. It is reasonable that a population study of bacteriuria should cover many years before valid conclusions can be drawn.

According to several investigators, bacteriuria should not cause renal scars after the age of four (13-15). However, when diagnosing scarred kidneys and vesico-ureteric reflux in 55- or 60-year-old women, it does not seem indisputable that they should have acquired these changes in early childhood.

The next examination of the present population sample is planned to take place as a follow-up in 1980-81. The indications for it will be discussed in a later communication. A report on the serological grouping of strains.

At present we restrict the conclusions to the commendation that bacteriuric women should be submitted to a concentrating test. A single defect, an unfavourable resistance pattern, bacteria *Proteus* and recurrent symptoms are indications for a pyelographic cystoscopy.

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Occurrence of Adult Fanconi Syndrome in Benign Monoclonal Gammopathy

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Adult Fanconi syndrome has been a manifestation of a latent form of myeloma, the benign monoclonal gammopathy (BMG). In a series of 10 patients with this form of myeloma we studied ten patients with respect to the adult Fanconi syndrome. These patients were selected from a larger series of BMG because of urinary excretion of light chains. In no instance could a complete Fanconi syndrome be shown, but one patient, with diabetic nephropathy, had very high urinary β -microglobulins. Two additional patients showed minor features included in the adult Fanconi syndrome. BMG does not seem to be associated with an increased risk of developing adult Fanconi syndrome.

Key words: Benign monoclonal gammopathy, adult Fanconi syndrome.

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The presence of urinary monoclonal light chains (Bence Jones proteinuria) in patients with some forms of plasma cell dyscrasia has been linked to impaired renal function (21). Consequently the renal function in these patients has been regarded as an important manifestation of the disease with relation to prognosis.

An increased amount of monoclonal light chains in the urine of patients with multiple myeloma may cause impaired renal function and secondary hypertension. It has been proposed that monoclonal light chains, especially those of kappa type, play a role in the development of renal tubular dysfunction, so that features suggestive of adult Fanconi syndrome appear (20). The adult Fanconi syndrome has been reported in patients with multiple myeloma, amyloidosis and Bence Jones proteinuria (7, 8, 11, 15, 16). In most reports the diagnosis of renal tubular dysfunction preceded the development of multiple myeloma or amyloidosis, never the reverse (8).

Patients with plasma cell dyscrasia and adult Fanconi syndrome represent a distinct variant of the monoclonal gammopathies, characterized by slow growth of the tumor with early development of metabolic complications, for example renal proximal tubular dysfunction (20). Adult Fanconi syndrome is defined as a dysfunction of the proximal renal tubules resulting in vitamin D resistant osteomalacia, glucosuria, generalized aminoaciduria, hypophosphatemia, chronic acidosis and hypopotassemia (16).

Since adult Fanconi syndrome has been described as preceding myelomatosis or accompanying plasmacytic dyscrasia with mild plasmacytosis, one would suspect that it could also be associated with benign monoclonal gammopathy (BMG), which is in essence a premyelomatous condition and not infrequently associated with excretion of monoclonal light chains in the urine (18). Therefore we decided to study a group of patients with BMG with respect to adult Fanconi syndrome. We chose to select those among 45 patients with BMG who—in addition to plasma M component—had Bence Jones proteinuria. We also thought that this could have therapeutic implications, perhaps one could identify a subgroup of BMG patients at risk, prone to develop renal proximal tubular dysfunction. These patients could then be treated in an earlier phase of their disease (1, 10, 22) to prevent excessive production of monoclonal light chains and subsequent tubular dysfunction.

PATIENTS

We studied 10 patients, 6 men and 4 women, mean age 74 years (range 62-88), classified as having BMG. The criteria for classification are published elsewhere (18). All ten patients excreted light chains in the urine in various quantities (Table 1). The duration of the disease varied from 9 months to 16 years (mean 4.5 years).

ponents showed a different electrophoretic pattern from those of the corresponding free denatured with the aid of immunoelectrophoresis as monoclonal kappa or lambda. In Bence Jones proteins were of kappa or lambda type. Calculated quantities of these protein varied from 60 to 570 mg/l (no. 5) excreted more than 7000 mg/l of immunoglobulin consisting of the V-component and a smaller amount of J-kappa chains. This patient also had mild diabetic nephropathy. Eight had low plasma concentrations of urinary background immunoglobulins.

Patients excreted increased amounts of amino acids in the urine while seven had normal values.

Monoclonal IgG had normal serum concentrations of phosphate and calcium. Four had a slight increase of albumin concentration. Serum concentrations of sodium and potassium were normal.

Renal function as defined as 24-hour excretion exceeding 1.5 ml/min was observed in two patients (one was a female).

One patient showed a urine pH below the range of 4-7. On the same occasion his serum uric acid concentration was 71 mmol/l (range 4-37).

DISCUSSION

It has been shown that prolonged excretion in the form of monoclonal light chains—particularly kappa chains—without evidence of definite multiple myeloma is characteristic of adult Fanconi syndrome. Experimental evidence (4) indicates that the defect is in the proximal tubule cells in the kidney. It is suggested that prolonged reabsorption in these cells of amino acids in the glomerulus. The kidney is the site of catabolism of light chains which was shown by Wochner et al. (25) who studied the fate

of radioiodinated Bence Jones protein in the humanized ureter of several mice. They found active and vigorous catabolism of light chains in overall Bence Jones metabolism. The catabolism of light chains accounted for less than 1% of the total metabolism. It is therefore suggested that prolonged overload of the kidneys with light chains may damage the cells responsible for catabolism which are most likely proximal tubular cells.

Several cases of adult Fanconi syndrome have been reported to occur in association with multiple myeloma (8, 16, 20). In all cases the disease has preceded the myeloma.

Since it has been shown that in the premyelomatous condition there is excretion of monoclonal light chains in the urine (14, 18) it could be suggested that patients are at risk of developing Fanconi syndrome. Maldon et al. (19) reported that patients with Fanconi syndrome and renal failure have a distinct pattern of amino acid excretion characterized by a low excretion of lysine. This type of slowly progressive proteinuria is also typical for BMG. It would be suggested that the same type of complication in Fanconi syndrome would also be found in BMG. For this reason we decided to study patients with BMG who also showed Bence Jones proteinuria with respect to Fanconi syndrome. Patients were selected among 45 with BMG. They were all essentially well with no concomitant disease except for one who had diabetes mellitus and diabetic nephropathy.

Our study shows that patients with BMG do not run a high risk of developing Fanconi syndrome. One patient (no. 5) showed incompletely developed Fanconi syndrome with excessive excretion of β_2 -microglobulin and aminoacids. However since this patient also has diabetic nephropathy (proven by kidney biopsy) it cannot be maintained that the Fanconi syndrome is due exclusively to the urinary excretion of monoclonal light chains. Available data (23) indicate that poor metabolic control in patients with diabetes sometimes may cause increased β_2 -microglobulin in the urine. We therefore believe that the partial Fanconi syndrome observed in this particular case has a dual etiology: diabetic nephropathy and pronounced excretion of monoclonal immunoglobulins as part of a BMG. This patient excreted kappa chains and complete IgG V-com-

ponent which was the major part of the excretion (Table 1). Two additional BMG patients had in complete Fanconi syndrome one of them (no. 7) had elevated levels of β_2 -microglobulin and blood urea and a decreased creatinine clearance the other (no. 6) had slight aminoaciduria, elevated blood urea and a decreased creatinine clearance.

Our conclusion is therefore that patients with BMG usually do not develop adult Fanconi syndrome. The reason for this is the usually low grade of Bence Jones proteinuria. Patients who have developed Fanconi syndrome in conjunction with plasma cell dyscrasia have usually had several grams of monoclonal light chains per liter urine. There are however exceptions to this general rule both BMG patients in our series who showed a partial Fanconi syndrome (nos. 6 and 7) had low grade Bence Jones proteinuria (60 and 190 mg/l respectively). The partial Fanconi syndrome in case 7 consisted mainly of markedly elevated excretion of β_2 -microglobulin while in case 6 this diagnosis was based mainly on a slight elevation of amino acids in the urine. Interestingly patient 6 excreted lambda type light chains which has not previously been described in conjunction with adult Fanconi syndrome.

From a therapeutic point of view we feel that treatment with cytostatics and corticoids is not indicated in patients with BMG even if they have a low grade (500 mg/l or less) Bence Jones proteinuria. But if the excretion exceeds that level and if the patient also shows evidence of Fanconi syndrome it would be logical to start such treatment since it could reduce the risk of permanent tubular cell damage.

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Lymph Node Toxoplasmosis

Follow up of 237 Histologically Diagnosed and Serologically Verified Cases

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ABSTRACT The clinical features, histology and follow-up of lymph node toxoplasmosis are presented in

of 237 histologically and serologically verified cases. Lymph node toxoplasmosis is a disease with nonspecific symptoms and in most patients the enlarged lymph nodes were the only sign. Three fourths of the patients were women and the majority were under 40 years of age. The clinical picture was not typical but suggestive features included a relatively enlarged lymph node, presence of the nodes in the neck and axilla, lymphocytosis in peripheral blood. Histologically, in the lymph nodes were characteristic features were strong hyperplasia of the lymphoid general structure with small groups of lymphoid cells both in the paracortical area and in the medulla. Strands of monocytoid cells were found. 80% of the cases with typical histology also had high antibody titers and in more than 50% of the cases with high antibodies, the lymph node presented a typical picture of toxoplasmosis. The follow-up revealed that lymph node toxoplasmosis is a disease without complications nor is there a connection with malignant lymphomas.

Key words: Lymph node toxoplasmosis.
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Toxoplasma gondii is an intracellular parasite that causes infections both in animals and man (5, 17, 23). The organism is widely distributed almost throughout the world. A significant number of people have antibodies against Toxoplasma as a sign of previous infection in Finland (1) and in the USA about 50% of the population (11).

The most serious but rare form of toxoplasmosis is congenital intrauterine infection, usually including encephalitis. Cerebral damage in the survivors is common. Fatal general infections have been reported in immunosuppressed and cancer patients. Rarely myocarditis, myositis or uveitis may be the only manifestation of toxoplasmosis.

Lymph node toxoplasmosis accounts for the great majority of infections caused by Toxoplasma gondii. The disease was first reported in Scandinavia by Gard and Magnusson (6) and later by other authors (8, 22). The usual diagnostic method has been lymph node biopsy and subsequent antibody determinations. Toxoplasmosis presents with special histological features in the enlarged lymph nodes. Lymph node toxoplasmosis is usually a mild disease (22, 25). Later development of Hodgkin's disease after a well documented case has, however, been reported (21) and a possible infectious etiology in Hodgkin's disease has been discussed (9).

The aim of this study was to assess lymph node toxoplasmosis and its prognosis in a larger material as well as its possible connection with later malignant lymphomas.

MATERIAL AND METHODS

Lymph nodes suspected of toxoplasmosis by a pathologist were collected from Finnish pathology laboratories during 1963-78. A total of 667 cases were found in which toxoplasmosis was suggested or mentioned as a differential diagnosis by the pathologist (2-4 of all lymph nodes from different laboratories). The slides were re-examined histologically without knowledge of the clinical or serological data. The previous histological criteria for toxoplasmosis suggested by Piringer Kuchinka et al. (14) and Saxen and Saxen (18) were employed.

Four hundred and three cases showed the typical histological picture of toxoplasmosis. Serology was available in 303 cases. In 237 cases the antibody titers were assessed high enough to admit the diagnosis of recent toxoplasmosis (dye test titers ≥ 256 , immunofluorescence (IF) titers ≥ 160 or complement fixation (CF) titers ≥ 16). These 237 histologically and serologically proved cases are analyzed in the following.

There were 23 additional cases with histology of toxoplasmosis but fully negative serology (dye test or IF < 4 or CF < 8). In seven cases there were high antibody titers without typical histology. To pick out cases with histology diverging from the usual 350 cases with positive serology

Abbreviations: IF = immunofluorescence; CF = complement fixation.

AGE (YEARS)

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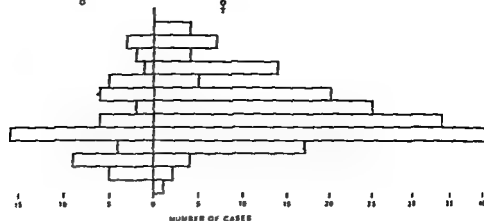


Fig. 1 Age and sex distribution of the 237 cases of lymph node toxoplasmosis

($\text{dys test} \geq 160$ or $\text{CF} > 8$) were checked for histology. In addition to the 111 cases of typical toxoplasmosis already included, three other cases were found. Two were non-specific hyperplasia, one was necrotizing granulomatous inflammation with positive tuberculosis culture. Histological differential diagnoses will be treated in a forthcoming paper.

The clinical data were obtained from the hospital charts and serology was checked from the serological laboratories. For comparison of the clinical data, 61 cases of non-specific hyperplasia with negative serology of toxoplasmosis were singled out from the basic material of 667 cases. The patients were followed on the basis of hospital records when available. In addition they were checked from the Population Registry of Finland for survival up to the end of 1977 and for possible later malignant lymphomas from the Finnish Cancer Registry up to the end of 1976.

RESULTS

The age and sex distribution of the 237 patients is presented in Fig. 1. Three fourths of the patients were women and three fourths were under 40 years of age. The youngest patient was a 5-year-old girl, the oldest a 63-year-old woman. The mean age of the men was 27.5 years and of the women 31.6 years. The cases were distributed all over Finland.

Symptoms. In the majority of cases the only symptoms were enlarged lymph nodes, usually noted by the patients themselves. The nodes were often initially tender and there was local pain in the adjacent structures. Physicians discovered enlarged lymph nodes in five patients during their follow-up for an unrelated disease. Transient fever had been noted in 38 patients (16%). Mild symptoms resembling virus infection (sore throat, tiredness and

muscle pain) were often reported in the charts. Three patients were hospitalized because of fever of unknown origin before the diagnosis. In five cases skin eruption was noted on the extremities. Muscle biopsy taken from one patient with muscle pains revealed myositis with small groups of epithelioid cells like those in lymph node toxoplasmosis. No other extranodal manifestations of toxoplasmosis were proved.

Probably most of the lymph nodes were enlarged to exclude the possibility of malignancy. All the patients had been treated with antituberculous effect. Only occasionally was toxoplasmosis suspected clinically.

Lymph node enlargements. The usual site (185%) was the head and neck region (Table 1). Nodes were often situated behind the ear.

Table 1 Location of the enlarged lymph nodes

	Toxoplasmosis (%) (n=234)	Non-specific hyperplasia (%) (n=61)
Head and neck		
Anterior neck	54	3
Posterior neck	27	
Supraclavicular	3	
Parotid region	1	
Axilla	11	14
Groin	3	6
Mammary glands	1	3
Other	-	
	(not evaluated in 3 cases)	

II Duration of the lymph node enlargement

	Toxoplasmosis (%) (n=168)	Non specific hyperplasia (%) (n=43)
Less than 1 week	11	9
One month	28	14
2-3 months	34	19
4-6 months	21	18
7-12 months	2	5
More than 12 months	4	35
	(not indicated in 49 cases)	

ideus muscle. Axilla, groin and the submammary glands were rare locations. The lymph node enlargements were reported as follows: in 25 cases (11% of cases with sufficient material for this respect). Chest X ray showed no changes in the pulmonary hilus (45 cases). X ray of the spine showed no changes.

On average the nodes had been noted for 4.5 months but for less than four weeks in 44% of cases (Table II). The majority of nodes were smaller than 3 cm in diameter (Table III).

Laboratory examinations. ESR was normal in 117 of the cases and marked elevation (>20 mm/hour) was noted in 10%. Leucocytosis was exceptional. Relative lymphocytosis was the rule. The number of lymphocytes in the differential count was 40% in 84% of the cases (Table IV). Eosinophils were not elevated. In six cases atypical lymphocytes were reported in the differential count. Anemia was exceptional.

Diagnosis. The antibody titers were usually measured for the first time soon after biopsy. Already at the first visit the titers were elevated in 216 (91%) of 237 cases. Whereas in 21 cases (9%) a fourfold rise was found in sequential measurements. Dye test/IF and CF correlated well in 80% of the cases, but in 20% the CF titers were low (≤ 8) when titer by IF or IF showed high titers (≥ 160). The exact interpretation of serological data is complicated by the different scales used in different laboratories. The Tunnell test for mononucleosis was negative or weakly positive in 30 cases, but in one case the titer was 1:224, probably due to a double infection (toxoplasmosis and mononucleosis).

Treatment. Specific treatment (pyrimethamine 25 mg 1-2 plus sulfonamides e.g. sulfadiazine 500 mg 4 times daily) was given to 29 patients (12%). In three

Table III Maximal diameter of the enlarged lymph nodes

	Toxoplasmosis (%) (n=114)	Non specific hyperplasia (%) (n=36)
Less than 1 cm	18	33
1-2 cm	50	50
2-3 cm	25	11
3-4 cm	1	6
More than 4 cm	1	-
	(not indicated in 123 cases)	

patients the treatment caused transient leucopenia. Abortion was performed in three women because of suspected threat from toxoplasmosis to the fetus.

Histology (Fig. 2, Table V). In the serologically confirmed cases the histological picture was rather uniform. The general structure was hyperplastic with abundant germinal centres and an active paracortical area. As a specific feature there were groups of epithelioid like cells with abundant slightly eosinophilic cytoplasm. Strands of mononuclear cells (unreife Sinushistiocytose of Lennert (12)) were usually found as well as paracortically located large lymphoid cells (immunoblasts). Giant cells of Langhans type, large granuloma necrosis and fibrosis were rarely found. Quite typically inflammatory infiltrate with lymphocytes and groups of epithelioid cells permeated the capsule. The parasite itself was not seen for certain.

Follow up. The symptoms had usually disappeared by the last visit to the doctor, about two weeks after the biopsy. In addition the patients

Table IV Relative proportion of lymphocytes in the differential count of blood leucocytes (range 0-100)

	Toxoplasmosis (%) (n=77)	Non specific hyperplasia (%) (n=27)
0-30 or less	4	22
31-40	12	33
41-50	47	19
51-60	23	11
61-70	13	7
71 or more	1	7



Fig. 2 Histology case of lymph node toxoplasmosis. In the corner there are centers with small epithelioid cells surrounding them of mononuclear type. Hematoxylin, original magnification.

were followed on the basis of later hospital records due to other diseases. 89 patients were followed for 1-16 years (average 4.9). All the patients were checked from the Population Registry and 235 were alive. The two deaths were not related to lymph node diseases or toxoplasmosis. No later cases of malignant lymphomas were found from the Finnish Cancer Registry (total follow up 1111 person years).

Table 1. Various histological features in the 237 proved cases of lymph node toxoplasmosis

	N	%
General structure		
Well preserved	235	99
Slightly distorted	2	1
Germinal centers		
Abundant	234	99
Sparse	3	1
Paracortical area well developed	216	100
Epithelioid cells in groups		
In the paracortical area	217	100
In germinal centers	218	92
Strands of mononuclear cells	205	87
Perinoditis	198	84
Lymphangioectasis	8	3
Necrosis	5	2

191 cases excluding the most recent ones (five years and ten months).

DISCUSSION

Toxoplasmosis comprises 5-15% of all lymph node enlargements on the basis of various studies (22-23). In our material of excised lymph nodes the percentage was 2-4. In an unselected material of lymph nodes have been found in 15-25% of the patients in Finland (7). Toxoplasmosis can thus be considered a common infection although not recognized.

Lymph node toxoplasmosis may manifest as enlarged lymph nodes as shown by the material collected among biopsied cases in a series of Tenhunen (21) and Räsänen (14). The clinical features of symptomatic patients were (10) respectively. In Tenhunen's study the association of the symptoms was however based on a questionnaire to the patients and the symptoms were invariably mild.

Clinical diagnosis can be aided by the fact that the majority of patients with lymph node toxoplasmosis are women, the nodes are located in the axilla and neck region and the duration of the lymph node enlargements is often less than one month. In

g. abnormality is quite constant relative to the lymph node hyperplasia. In contrast patients with non-specific lymph node hyperplasia have a longer history and relative lymphocytosis is less common. Mononucleosis shares many features with toxoplasmosis as previously noted by Lennert. Patients with Hodgkin's disease usually have general symptoms including weight loss, fever, and night sweats. Blood tests before therapy often show leukocytosis with granulocytosis and lymphopenia (13). The clinical features in toxoplasmosis are not specific, however, and to obtain a definite diagnosis an enlarged lymph node is excised. Histological features in lymph node toxoplasmosis are rather specific, judging from this material of 203 cases with typical histology. 237 had positive antibody titres. The specificity of the histological picture is thus about 80% or even higher. In many cases serological examination was performed before the rise of titers (19). The correlation between the histological picture and high toxoplasma titers has been shown by the authors (4, 14, 20, 24). The sensitivity of the histological diagnosis is also high, because only 7 of 246 seropositive cases in this study presented a non-specific histology. The histological differential diagnosis will be treated in a forthcoming paper. The final diagnosis is made on the basis of histological investigations. For differential diagnosis and possible therapeutic implications a serological diagnosis is needed. When negative in the lymph node, the antibody titers should be determined repeatedly. The parasite can be isolated from the lymph node, but this is not always possible and was not done in the present cases. Specific treatment is not required in lymph node toxoplasmosis, but is reserved for the most symptomatic cases. Previous lymph node toxoplasmosis does not cause any damage, but infection during pregnancy is hazardous to the fetus (3, 25). The source of toxoplasmosis cannot be determined on the basis of histology. Epidemiological studies have revealed a significantly higher percentage of patients who are consumers of raw meat than among controls. One epidemic of five patients was probably due to contaminated hamburgers (10). Recently the ingestion of *Toxoplasma* trophozoites through oral contact with a membrane has been demonstrated experimentally in the rabbit (16). This could also explain the mainly cervical location of lymph node toxo-

plasmosis. Cats have formerly been considered a source of toxoplasmosis and also epidemics have recently been reported (27).

The follow-up of the present cases based on the Cancer Registry data suggests that toxoplasmosis bears no relationship to malignant lymphomas; the relationship observed by Sheagren et al. (21) must have been co-incidental. The Cancer Registry has previously, on the basis of check-ups, proved reliable when tracing malignancies (26). The life expectancy of the patients does not differ from that of the general population.

Toxoplasmosis should be considered in the clinical differential diagnosis of lymph node enlargements, especially in young and female patients with cervical adenopathy and relative lymphocytosis in the peripheral blood.

ACKNOWLEDGEMENT

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Angiotensin Converting Enzyme in Newly Detected Sarcoidosis

With Special Reference to Erythema Nodosum in Patients with Erythema Nodosum

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ABSTRACT Serum angiotensin converting enzyme (ACE) was measured in 56 patients with sarcoidosis three months after diagnosis. Serial analyses were performed in 42 patients who were followed for months to one year. SACE was elevated in 49% of the patients at some time during the period of observation. Erythema nodosum (EN) was present in 14 patients. Only one out of seven had elevated ACE during EN, but four months later SACE was elevated in all 14. In this group there was a negative correlation between SACE and S-lysozyme. Among patients without EN the frequency of SACE elevation was rather constant, about 60%. In half of the patients SACE was normal at first examination and subsequent elevation was observed in 19%. SACE and S-lysozyme were positively correlated. Enzyme activity was independent of mode of presentation, but a significant trend toward higher SACE in patients with pulmonary involvement was observed. Changes in SACE were roughly correlated to radiological changes. Among healthy controls significantly higher SACE levels were found in children up to 17 years of age (reference interval 21.2-141 U/ml) than in adults aged 18-65 years (12.0-36.8 U/ml). The observations on patients with sarcoidosis demonstrate that a unique pattern of SACE occurs in patients with EN, different from that of patients without EN. The findings support the view that EN is a marker of acute-onset sarcoidosis. The value of serial SACE analyses is emphasized.

Key words: angiotensin converting enzyme, lysozyme, sarcoidosis, erythema nodosum, age variation.

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Since the first extensive study on serum angiotensin converting enzyme (SACE) in sarcoidosis (9), elevated enzyme levels in patients with this disease have been noted by several investigators with a frequency ranging from 29 (25) to 88% (9). This is independent of method and may be due to

the composition of series with different proportions of active and inactive cases.

Among cases of newly detected sarcoidosis (i.e. subacute) elevated SACE has been found in 45-60% (7, 24, 25), but the figures are difficult to interpret because examination occurred at different times in the course of disease.

The aim of the present study was to examine SACE in newly detected sarcoidosis with special reference to clinical mode of presentation. Furthermore, a reference group of children is presented.

SUBJECTS

The series consisted of 56 consecutive patients with newly detected sarcoidosis during a two-year period in 1977-79.

The diagnosis of sarcoidosis was made without knowledge of the SACE level and was stated according to clinical and roentgenological criteria (18, 20). Non caseating epithelioid granulomas were found in 71 patients. Haematological, bacteriological and serological investigations were performed in all patients in order to exclude the possibility of tuberculosis, yeastosis, other infectious diseases and malignant disorders. In 54 patients the first SACE analysis was performed within two months after the diagnosis of sarcoidosis and in two patients after three months.

The patients were divided into two groups according to presence or absence of erythema nodosum (EN) (Table II). Only one SACE analysis was performed in 14 patients (among them one with EN). 17 analyses were performed in 47. The follow-up time was 3 months to 1 year (mean 9 months).

In the following time intervals the onset of EN in patients in whom a distinct debut could be assured, as regards patients without EN, indicates the point of time when the first signs of sarcoidosis appeared. The first abnormal chest X-ray (CXR) suggesting the disease.

As adult controls we used 116 healthy persons aged

Abbreviations: ACE = angiotensin-converting enzyme; SACE = serum ACE; CXR = chest X-ray; EN = erythema nodosum.

Table 1 Sex and age distribution of the 56 patients

	N	%	Males	Females	Age (y)	
					Mean	Range
With EN	14	25	7	7	28	19-48
Without EN	42	75	25	17	31	16-66
Total	56	100	32	24	30	16-66

18-65 years (14). In view of recent reports on age variation of SACE (13) 86 non sarcoid children aged 5 months-17 years were examined (17). The male:female ratio was 40:46.

METHOD

SACE was analysed according to Cushman and Cheung (1) as modified by Lieberman (9). SACE activity was expressed in U/ml equivalent to nmoles of hippuric acid liberated per ml per min.

Serum lysozyme was determined immunochemically according to the method of Laurell (Medicinsk Laboratorium, Copenhagen). Reference interval 0.08-0.32 μ mole/l.

The cumulated frequency of elevated SACE was calculated in the following way:

$$\frac{\text{No. of pats. with elevated SACE during a given period} \times 100}{\text{Total no. of pats. examined during the same period}} \%$$

For example: at time 0 months 14 out of 20 patients examined had elevated SACE = 70%. At 1 month 5/16 had elevated SACE = 31%. At this time a total of 32 patients had been examined. 16 of these 32 had elevated SACE giving a cumulated frequency of $16/32 = 50\%$. At 2 months elevated SACE had been found in 20 out of 39 patients examined during time 0-2 months giving a cumulated frequency of 51% etc.

Statistics (14)

Differences between mean values were calculated using Student's *t* test in small subgroups using the Mann-Whitney rank sum test. Difference of frequency was calculated with χ^2 test and correlation with Spearman's rho. Significance level 5%.

RESULTS

Controls

Fig. 1 shows a significant difference ($p < 0.01$) between SACE in persons up to 17 years of age (SACE 32.2 ± 5.5 U/ml (mean ± 1 S.D.)) and adults aged 18-65 years (SACE 24.4 ± 6.2 U/ml). Thus the reference interval (mean ± 2 S.D.) was 21.2-42.2 and 12.0-36.8 U/ml respectively. Fifteen (17%) of

the children had SACE above the upper limit for adults. No sex difference was noted.

Sarcoidosis patients as a whole

Considering all 56 patients, elevation of SACE observed in 15 (26%) out of 27 at time 0 and 39 patients (70%) had elevated SACE when served for up to one year. Among 21 patients whom non caseating epithelioid granulomas detected elevated SACE occurred in 14, the mean SACE 45.5 U/ml. Among 35 patients in whom no tissue biopsy was performed the corresponding figures were 21 (60%) and 47.4 U/ml. The difference was not significant. No relationship was found between SACE and systemic blood pressure.

Sarcoidosis without erythema nodosum

Among the 42 patients without EN 11 (26%)

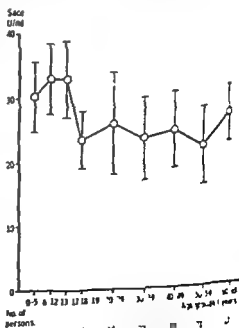


Fig. 1 SACE levels of the control subjects vs. decrease in persons over 18 years of age compared to those 17 years of age or younger.

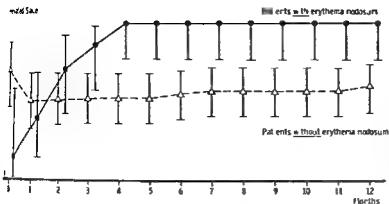


Fig. 2 Cumulated frequency of elevated SACE including 95% confidence limits in patients with and without EN

and SACE (mean 41.4 ± 16.4 U/ml) at first examination at 0-3 months which is significantly higher than in the controls ($p < 0.001$). Twenty patients were examined at time 0-14 (70%) of these elevated SACE.

Of 19 (19%) of 21 patients with normal SACE at examination developed an elevated enzyme during the time of observation giving a total frequency of SACE elevation of 25/42-60% (95% confidence limits 43-74.4) as demonstrated in Fig. 2. Mean SACE in these patients displayed fluctuations with no consistent pattern.

Among the patients with a persistently normal SACE at serial analyses deviations of more than 20% were noted in five: two with increasing and three with decreasing SACE.

The highest SACE observed was 116.1 U/ml (maximal SACE was 44.2 ± 20.0 U/ml).

Twenty-three patients were admitted because of symptoms of disease and 19 were detected on chest CXR. The frequency of SACE elevation at examination was the same in both groups: 52/77% respectively.

As shown in Table II a trend towards a higher mean SACE and a higher frequency of elevated SACE was found in patients with CXR stages II+III (only one patient had stage III) than in patients with CXR stage I but the difference was not significant.

The course of the disease in relation to SACE levels is demonstrated in Table III. No consistent pattern could be found. But among 30 patients with a regular CXR follow-up regression was noted in 12 (40%)—in three patients in spite of increasing SACE. Only one patient showed progressive lung changes.

Serum lysozyme was analysed in 20 patients. Fig. 3 demonstrates a positive correlation between SACE and serum lysozyme ($p < 0.001$). Among 33 paired values an elevated serum lysozyme despite a normal SACE was noted in 11 (33%) whereas both SACE and serum lysozyme were elevated in 19 (58%). In no instance was there elevated SACE despite a normal serum lysozyme.

Among patients with normal SACE at first examination 12/19=63% were tuberculin negative.

Table II *Serum angiotensin converting enzyme (mean \pm S.D.) related to chest X-ray in 42 patients with sarcoidosis without erythema nodosum*

		SACE at first examination					
No of pats	U/ml	Pats with elevated SACE		Maximal SACE (U/ml)	Total frequency of elevated SACE		
		n	%		n	%	
20	38.0 ± 14.3	9	45	43.2 ± 20.5	11	55	
22	42.9 ± 18.1	12	55	45.0 ± 17.9	14	64	
42	41.4 ± 16.4	21	50	44.2 ± 20.0	25	60	

Table III Relationship between *S* angiotensin converting enzyme and chest roentgenotuplet 42 patients with newly detected sarcoidosis observation period 2-12 months

Course of SACE	Course of CXR changes			
	Progression	No change	Regression	Total
<i>Patients with EN (n=14)</i> (only a single analysis performed in 1 patient)				
Increase	4	5	3	12
No change	0	0	0	0
Decrease	0	0	1	1
Total	4	5	4	13
<i>Patients without EN (n=42)</i> (only a single analysis performed in 12 patients)				
Increase	1	8	3	12
No change	0	3	2	5
Decrease	0	6	7	13
Total	1	17	12	30

compared with $13/16=81\%$ among patients with elevated SACE. This difference was not significant.

Sarcoidosis with erythema nodosum

EN was the first sign of sarcoidosis in 14 patients; in eight cases preceded by arthralgia or arthritis for days to weeks. SACE was examined during EN (time 0) in seven of these patients. After one month SACE had been analysed in 13 patients and after two months in all 14.

Fig. 2 shows that only one (14%) out of seven patients examined during EN had an elevated SACE while it increased gradually in 13 during the following weeks to months; in one patient as late as after four months at which time SACE had been elevated in all patients. This frequency of SACE elevation was significantly different from that noted in patients without EN as estimated by confidence limits ($14/14=100\%$; 95% confidence limits 76.8-100). Mean SACE is shown in Fig. 4 where a continuously normal SACE in three patients with non-sarcoid EN is shown for comparison.

In one patient only a single analysis was done. Significant changes in SACE occurred in all others; as a rule an increase. This course was in contrast to that of patients without EN. In the patient with elevated SACE at time 0 SACE began to decline after four months simultaneously with regression of pulmonary infiltrations.

There was no relationship between the stage of CXR and SACE (10 patients had stage I and 4 stage

II). The relationship between the course of CXR and SACE levels is shown in Table III. An increasing SACE was not followed by a corresponding pattern of changes in CXR. It should be noted that the only patient with a decreasing SACE had concomitant roentgenological regression.

Maximal SACE was 53.9 ± 12.8 U/ml, significantly higher than the corresponding value for patients without EN ($p < 0.05$).

In contrast to patients without EN all

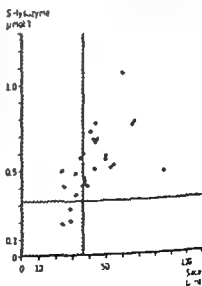


Fig. 3 Correlation between lysozyme and SACE in 20 patients with sarcoidosis. $Rho = 0.02$, $p < 0.001$.

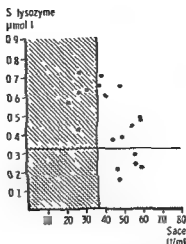
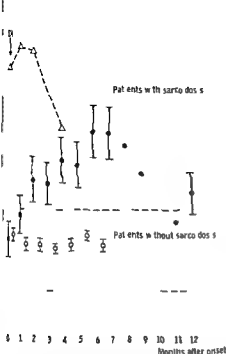


Fig 5 Correlation between 19 corresponding values of serum lysozyme and SACE in 6 patients with sarcoidosis and EN. $Rho = -0.512$, $p < 0.05$.

Fig 6 SACE (mean \pm S.D.) in 13 patients with sarcoidosis and EN. SACE of an additional patient with sarcoidosis during EN is shown separately. For SACE in three patients with non-sarcoid EN, see text.

between SACE and serum lysozyme was observed in patients with EN ($p < 0.001$) (Fig. 5). Serum lysozyme was uniformly elevated during EN in all patients examined and decreased subsequently while SACE increased typically for 2 months after EN. The course of a representative patient is shown in Fig. 6. Of 13 patients, 12 (92%) were tuberculin negative and no relationship was found between SACE and tuberculin reactivity.

DISCUSSION

Lofgren's classical study on subacute sarcoidosis (11) has been realized that sarcoidosis with EN in many ways behaves in a special manner, giving a most favourable prognosis and particular immunological features, e.g. a relatively high frequency of circulating immune complexes (7). These cases are also unique because the onset of disease can be established with relative certainty (10).

The present study presents another characteristic feature of sarcoidosis with EN, i.e. these patients have usually a normal SACE at the onset of EN—despite concomitant presence of epithelioid granulomas and tuberculin negativity—followed by a distinctly increasing SACE. This finding is in contrast to the situation in patients without EN, who have a relatively stable frequency of SACE elevation (60%) and supports the concept that EN is in fact an expression of the onset of sarcoidosis. It was also remarkable that serum lysozyme was elevated during EN in all sarcoidosis patients examined, with a subsequent decline and a negative correlation to SACE—in contrast to patients without EN. This phenomenon may be a clinical correlate to the experimental findings of Lieberman et al. (10). They induced lung granulomatosis in rabbits with a complete Freund's adjuvant and noted an increasing SACE after three weeks. On the other hand, serum lysozyme was elevated after only two days with a peak after three weeks.

The total frequency of elevated SACE of 70% in newly detected sarcoidosis is higher than that of 49% reported earlier (14). The cause of this seeming discrepancy is in part that serial analyses will unveil a larger number of enzyme elevations. Another reason is that patients with subacute sarcoidosis in our previous study had a duration of disease up to two years at the time of blood sampling. In some of these patients, therefore, a previous SACE elevation may have occurred before.

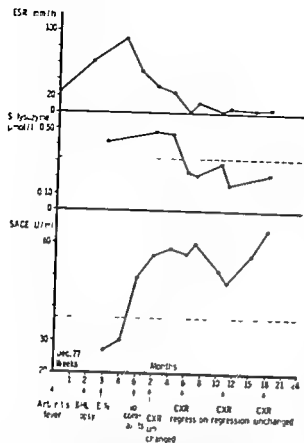


Fig 6 Course of SACE, serum lysozyme, ESR, and clinical signs in a 31-year-old male with sarcoidosis presenting with arthritis and EV. BHL = bilateral hilar lymphadenopathy.

examination. However, in spite of serial analyses, the frequency of enzyme elevation in most of the patients in the present series was not as high as the 85% reported in patients with an active disease of more than two years' duration (14).

Even if a patient's SACE is within the normal limits at several examinations, a rise or fall within these limits may occur, suggesting disease activity. Such fluctuations were observed in five patients with sarcoidosis, whereas similar analysis in normal persons showed a stable SACE for weeks and months.

Other investigators have found that SACE is higher in patients with roentgenological lung involvement than in patients with CXR stage I (16). Although the present series shows a trend against this, the difference is not significant. In fact, CXR gives only a rough impression of pulmonary involvement. Thus, Rosen et al. (19) found lung granulomas in all of their 21 patients with CXR stage I (isolated hilar or paratracheal adenopathy

without pulmonary infiltrations). This is in accordance with the view that sarcoidosis—even when the only manifest sign is adenopathy—is a multisystem disorder, demonstrated by the relatively high frequency of clinically inert sarcoidosis of the spleen and liver (18).

The clinical course of sarcoidosis during a period of observation was roughly correlated with levels of SACE. Progressive intrathoracic involvement associated with decrease in SACE were observed but roentgenological regression was seen in six patients despite increasing SACE.

The cause of SACE elevation in sarcoidosis is still unknown, although experimental and clinical observations point to the mononuclear macrophage cell line as a primary source of SACE in this disease (3, 10, 22). In addition to blood and epithelioid cell granulomas, increased ACE has been demonstrated in alveolar macrophages in patients with sarcoidosis (3). Although SACE is useful in diagnosis and follow-up of the disease, the pathophysiological significance is unclear. Thus, no correlation has been found between systemic blood pressure and SACE in other series (9); neither has a correlation been found between plasma renin activity and angiotensin in sarcoidosis (26). Involvement of the renin-angiotensin system in sarcoidosis is therefore unlikely. However, the other physiological function of this enzyme is bradykinin degradation (kininase II), and perhaps the elevated SACE in sarcoidosis reflects an involvement of kinin metabolism in this disease, has the character of a chronic inflammation. The different course of serum lysozyme and SACE in patients with EV suggests that lysozyme and ACE are produced from different cell lines, more probably from different stages in the development of epithelioid cells. Another observation is the age variation of SACE in normal persons—with a higher level in children and young adults. This finding is in agreement with other publications (5, 13).

It has been stated that other ACE assays (on other substrates or by spectrophotometry) (24) should be more convenient and specific than the present one. However, with respect to sarcoidosis, the frequency of enzyme elevation was the same magnitude in most series, independent of method. Furthermore, the present assay has already been very precise, with a coefficient of variation

003-007 (15) The elevation of enzyme seems to be rather specific for sarcoidosis. The positive rates ranging from 88 (14) to 94. A recent survey from our laboratory (16) had elevated SACE in only about 15% of 778 sarcoid and non sarcoid patients examined.

ACKNOWLEDGEMENT

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Prodromal Ventricular Premature Beats Preceded by a Diastolic Wave

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TRACT In a prospective study of quinidine syn-
drome patients with atrial fibrillation/flutter were
monitored for four days after the start of
therapy. Six patients developed ventricular
cardiac (VT) or ventricular fibrillation (VF) dur-
ing the first 48 hours in sinus rhythm. Q-T prolonga-
tion as seen in most patients with and without VT/
VF was non predictive in this respect. However,
a diastolic wave (DW) of larger amplitude than the
preceding T wave and usually followed by a ventricu-
lar premature beat (VPB) appeared in five of the six
patients (13-185 min before the first event).
In none of the remaining 63 patients reaching
sinus rhythm. For comparison, two of our own cases
came from the literature with idiopathic long Q-T
alternating T wave syndromes were studied for
DW-VPBs. They were found in all ten cases
of VPB recording, but among 19 patients with
myocardial infarction and VT/VF no such case
was seen. As VT/VF was more common after large
VPB after small amplitude DWs, it is discussed
whether the DW not only precedes but also initiates
ventricular tachyarrhythmias.

**Prodromal syndrome, long Q-T syndrome, diastolic
T wave syndrome.**

Acta Med Scand 208: 445-1980

A well known paradox that quinidine, although
sometimes used to prevent ventricular tachyar-
rhythmias, in other cases may produce ventricular
tachycardia (VT) or ventricular fibrillation (VF). It
has been suggested that these quinidine induced
tachyarrhythmias represent a form of the Q-T pro-
longation syndrome and that the Q-T interval
should be monitored in patients receiving quinidine.
A retrospective study showed however that
Q-T prolongation is an unspecific predictor of these
arrhythmias (5).

It has therefore been suggested to study, in a prospective
study, all ECG changes preceding VT and VF by

continuous monitoring during the first days of
quinidine therapy. Patients with idiopathic long
Q-T syndrome (24) and the related alternating T
wave syndrome (18-21) were also studied for com-
parison, as were patients with acute myocardial in-
farction (AMI) and VT/VF.

PATIENTS AND METHODS

Quinidine VT/VF

This prospective study (1976-78) comprised 72 patients
35 females and 47 males aged 33-83 years (mean 61). They
were hospitalized for conversion of atrial fibrillation
(64) or flutter (8). The etiology was apparent in only
9 cases: valvular heart disease, 9; ischemic heart disease,
6; systemic hypertension, on 5; treated thyrotoxicosis, 4; in
cohort, 3; cor pulmonale, 2.

All patients had been treated with digoxin but in 60
cases this was withheld 2-14 days before the conversion
attempt. Quinidine sulphate was given orally in a load-
ing dose of 0.01 g/kg body weight and a maintenance dose of 0.6
g (dumiles) started one hour later, every 12 hours. One
patient developed an allergic reaction after the loading
dose and was excluded from the series.

Thirty-four patients converted to sinus rhythm within
15 hours after the first dose of quinidine; after that, the
interval 35 of the remaining 37 patients were DC-converted to
sinus rhythm by maximally 75-300 Wsec (mean 169).

All patients were ECG monitored for 94 hours from the
start of quinidine therapy by a rhythm lead corresponding
to CR₂. The cable ECG was also recorded continuously
at a paper speed of 10 mm/sec by an ink jet recorder.
The recordings were evaluable for 95% of the time. A
12-lead ECG was recorded twice daily during these four
days. The Q-T interval was measured as the mean of two
cycles in lead II of the 12-lead ECG recorded about 24
hours after the start of quinidine treatment. The mea-
surement was performed only in the 34 patients in whom
both observers considered the Q-T end point distinct.
The upper normal limit was according to Lepeschek (16).

Abbreviations: VT=ventricular tachycardia; VF=ven-
tricular fibrillation; VPB=ventricular premature beat;
DW=diastolic wave; AMI=acute myocardial infarction.

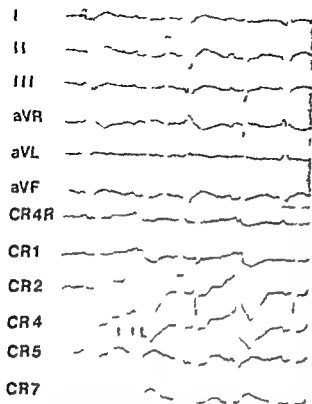


Fig. 1 A 12-lead ECG from a patient with quinidine-induced VT showing a diastolic wave (between the first two arrows) preceding a VT. One large square represents 0.1 sec and 0.5 mV.

Idiosyncratic QT syndrome

This group included the only patient with idiopathic long QT syndrome in our files and eight cases found in the literature with a good recording of the tachyarrhythmia (7/9 (11/17/0/33)).

Our patient was a 43-year-old man with a history of many episodes of dizziness and palpitations, especially when running uphill and during emotional stress. He had also had two syncope, and on these admissions the ECG showed VT, which was not of the wide depolarized type. The Q-T interval was prolonged during periods of ventricular ectopy beats but not otherwise. For seven years now he has been almost completely free from attacks of dizziness on β block therapy.

Alimentary T wave syndrome

This group includes the only patient with alternating T wave and VT/VT in our files and the only case found in the literature with a recording of the tachyarrhythmia (1).

Our patient was a 19-year-old woman with a slight digoxin intoxication (3.3 nmol/l serum) when she needed an acute operation with a valve prosthesis because of aortic aortic incompetence (19). On the day of operation she developed VT; on the next day a long VT of torsade de pointes type was recorded. The Q-T interval was pro-

longed during the first postoperative day. She had not had any history of syncope and had not taken any drug known to affect the Q-T interval.

Acute myocardial infarction

For comparison rhythm strips (Fig. 19 AMI) VT/VT were studied. None were considered as complications, but not as VT cases and patients with VT diagnosed by fusion beats.

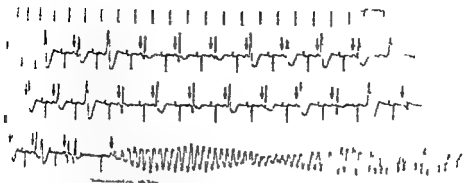
RESULTS

QT interval/VT/VT

Of the 71 patients, four developed VT on one or more occasions during the sinus rhythm. When long enough to be measured, it was often (63/111) of the type. No patient was known to have had VT before. There was no difference between with and without VT/VT with regard to concentrations of potassium, creatinine, digoxin, and even the interaction between digoxin concentration following quinidine in the two groups (4).

In 34 of the 71 patients the T wave had ended point so that the Q-T interval could be measured. In 87% of them the Q-T interval was prolonged, including all five measurable patients, and the five were scattered among others, i.e. the specificity of Q-T prolongation as a predictor of VT/VT was very low. An example in the ECG showed a good prediction of the diastolic wave (DW) of greater amplitude than the preceding T wave (Fig. 1) which, except as in a, followed by a very premature beat (VIB). The DWs occurred in 14 patients with VT/VT and in none of the remaining sinus rhythm. After 3-47 sec in sinus rhythm the DWs first appeared (mean 55) before the first episode of VT. The same time as the DWs, frequent VIBs usually in a big cm pattern.

In Fig. 1 the DW follows every VIB, the best seen in leads CR₂ and CR₇ before the two arrows. The amplitude increases from the first arrow until a premature, broad QRS complex arises at the second arrow. The third arrow denotes where the next VIB would have started, and it is apparent that it has a large amplitude. In a, the VIB cannot be the initial part of a QRS complex as that would mean a QRS



A rhythm strip (corresponding to lead CR₂) showing small QRS complexes (arrows) followed by VPBs and by VT of torsade de pointes type in a patient on

quinidine. The recorded interval is 0.5 sec. One large

0.3 sec which is not reasonable. Nor is it a pure P wave superimposed on the T-U wave. As the P duration would then be about 0.3 sec. The DW ought not to be called a giant U wave as that term has been used for moderately large amplitude than the T wave of the same

Fig 3 shows how wide premature QRS preceded DW starts after a long R-R interval caused by a premature P. The bigeminy goes on for about 1 min and turns into VT of torsade de pointes type on VF.

Fig 3 shows that DWs of small amplitudes are caused by single premature beats or couplets and of large amplitudes by VTs. This seems also the case in Fig 2 although less marked. In three of the five patients the phenomenon was as in Fig 3.

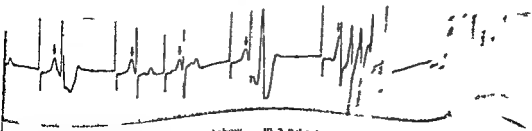
The VPBs and the first beat in VTs and VF had varying configurations in each patient and

the DW varied too. There was no fixed relation of DW-VPB configurations.

As rare exceptions DWs were initiated by VPBs. Sometimes there was a pause after the DW although the latter was a continuation from the sinus beats. Occasionally the DW was not followed by a premature beat and that appeared to happen especially just after a VT or VF had stopped. In these situations the DWs could show a 1:1 pattern all the time in rhythm although every second DWs had a larger amplitude.

Fig 4 shows a spontaneous DW in a patient on quinidine. The DW was initiated by a premature beat (100 W) and the cycle was cancelled (100 W). At first VPBs appeared by DWs in a 1:1 pattern and then by short VT.

In the literature eight cases of good recording of the tachycardia



A rhythm strip (corresponding to lead CR₂) showing small QRS complexes (arrows) of small amplitudes by VT by VPBs and waves of large amplitude by VT

in a patient on quinidine. The recorded interval is 0.5 sec.

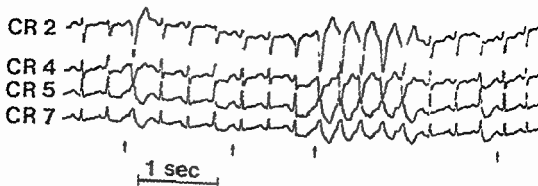


Fig. 4 Chest leads recorded 1 min post maximal bicycle exercise (300 W) for 1 min in a patient with idiopathic long

Q-T syndrome. Diastolic waves (arrows) followed by VIB or VT and sometimes not.

12 17 20 23). In five cases a DW can be seen to precede the first beat of the VT/VF (7 12 17 20 23) and in three there are DWs ahead of the VPBs (2 9 11) as in the other four with a recorded VPB (7 12 17 23).

Alternating T wave syndrome

In our case the ECG showing alternating T waves and VPBs in bigeminy or quadrigeminy (Fig 5 top) was recorded immediately after a VT of torsade de pointes type. Both the T wave alternation and the VPBs were suppressible with lignocaine (Fig 5 bottom) and isoprenaline (19). Fig 5 shows from comparison of the two panels that the T alternation is caused by a wave superimposed from the apex of the T wave onto the P wave. When this DW has a larger amplitude as in the first and last complex in Fig 5 top it precedes a VPB but when the amplitude of the DW is smaller as in the middle complex no VPB follows it. The only case found in the literature showed DWs preceding both VPBs and VT (1).

Acute myocardial infarction

None of ten AMI patients with VT showed a DW. In one of nine AMI patients with VF it was debatable whether the second and following beats were preceded by a DW but the first one was definitely not and there were no prodromal VPBs.

DISCUSSION

DWs followed by VPBs can also be seen in published cases of quinidine syncope (10 13 14 22). This prospective series demonstrates their role as a

highly sensitive (5 of 6) and specific (5) factor of VT/VF during quinidine therapy. A prolonged Q-T interval was much more common in the DWs and had a very low predictive value whereas DWs followed by VPBs can be prodromal. Their onset is easily controlled by prophylactic measures. The impending VT to be preventable by lidocaine prophylaxis are used (150 mg as bolus + 4 mg/min) in patients below 60 years, 100 mg + 3 mg/min in patients above 60 years (5). According to the ECGs in 19 and our own DWs followed by VPBs are prodromal also in patients with idiopathic and alternating T wave syndromes and the relationship between the DWs and the arrhythmias still more interesting.

The DWs and the VPBs usually appear separately and then occur together and disappear at the same time (19). A DW usually preceded by DWs of larger amplitude and VPBs. For these reasons it is probable that a DW not only precedes but initiates VIB. This hypothesis is not contradicted by the fact that a preceding DW was not seen in one of the VT/VF patients as the monitoring lead was ideal compared to the 12 lead ECG for DWs.

A recording published by Garza et al (2) supports further the hypothesis of a causal relationship between DWs and ventricular tachycardia. They provoked VF by epinephrine infusion in a brain damaged patient with a long QT syndrome. Studying that ECG one can see how a DW successively separates itself from the T wave and increases its amplitude until it be-

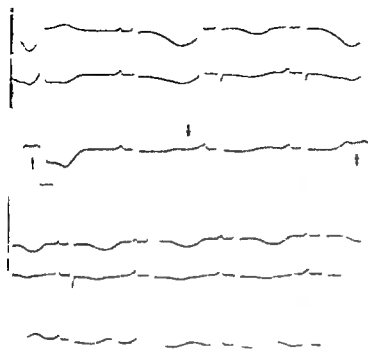


Fig 5 Extracardiac conduction after a VBI in a patient with alternating T wave after cardiac surgery. Sinus rhythm was preceded by a P wave (1). When the atrial T wave was not followed by a VPB (2) it is not followed by a T wave. The alternating pattern is apparent by comparing the QRS-T complexes 3 and 4. The alternating T wave is not followed by a T wave. The alternating T wave is not followed by a T wave. The alternating T wave is not followed by a T wave.

the R wave (lead II) and begins to be followed by VPBs and finally VF. These findings in the surface ECG are supported by recent report by Lazzarini et al (15) on ventricular electrograms (1 cm apart) in a case of Q-T syndrome. They found especially in the parts of both ventricles slow potential following the T waves. These waves in increased amplitude during infusion of epinephrine when they became high they were followed by a T wave. It is not known if the surface ECG simultaneously showed any DWs. The authors suggest that normal afterpotentials cause both the Q-T syndrome and during adrenergic stimulation tachyarrhythmias of the long Q-T syndrome. DW may also correspond to the second deflection of the right ventricular monophasic action potential described by Gavrilescu and Luca (8). The effect on was recorded in some catheter position in cases of congenital long Q-T syndrome at any site in a third case of the quinidine syndrome. It is less clear whether the basis of slow potential waves and the DWs are the same. The tendency of the VPBs to appear in a tachyarrhythmia gives some support for the idea

In conclusion in patients in whom the T wave is followed by VPBs are a highly sensitive and specific predictor of VT/VF within the nearest minutes to hours. The Q-T prolongation is non predictive in this sense. This probably also holds true in patients with idiopathic long Q-T and alternating T wave syndromes.

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24-Hour Blood Glucose Profiles in Insulin-Dependent Diabetics Treated with Intravenous Insulin Infusion Systems

A Comparison between Closed and Open Loop Systems

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ABSTRACT The aim of this study was to compare 24-hour blood glucose profiles of insulin-dependent diabetics during treatment with preprogrammed delivery systems with those of patients on treatment with the artificial beta cell (Biostrator[®]). Mean blood glucose (MBG) was 4.4 ± 0.5 mmol/l for patients on closed loop systems and 5.8 ± 1.0 for 53 patients on open loop and 4.6 for 20 patients on closed loop treatment. MBG was significantly higher in diabetics than in non-diabetics but the difference between the two populations was not significant. Mean amplitude of blood glucose excursion was 1.8 ± 0.6 mmol/l and 3.5 ± 0.8 mmol/l respectively, the difference between the diabetic groups being significant. Hypoglycemia was seen in patients during closed loop treatment whereas this was the case in 10 of 53 patients on treatment with open-loop systems. Physiological glucose fluctuations resulted in small but normal decreases in plasma insulin without hypoglycemia. Plasma insulin was within the normal range during both regimes. It was concluded that blood glucose control during treatment with closed loop system is superior to that during treatment with open loop systems. However, under standard conditions intravenous insulin infusion with programmed pump devices resulted in blood glucose fluctuations comparable with those during closed loop treatment.

Keywords: artificial beta cell, open-loop system, closed loop system, plasma insulin, insulin-dependent diabetes.
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Subcutaneous insulin therapy combined with restricted food consumption has not normalized the blood glucose of insulin-dependent diabetics. The morbidity among insulin-dependent diabetics exceeds that of age and sex matched non-diabetics

by about 600% (11). In western countries this depressing prognosis is caused by development of nephropathy, myocardial infarction and vascular cerebral insults, complications which beside retinopathy and neuropathy seem to be the result of deficient restoration of metabolic and endocrine abnormalities in the patients.

Permanent restoration of normal metabolic and hormonal conditions in diabetics without endogenous insulin secretion is extremely difficult because: 1) The postprandial plasma insulin peak in normals cannot be fully imitated even by subcutaneous injection of regular insulin (Fig. 1). 2) The reproducibility of absorption of insulin suspensions from subcutaneous tissue is too low (coefficient of variation 26%) (17). 3) Exercise increases the subcutaneous blood flow and thereby absorption of insulin (18). 4) Regular life with planned meals (which is necessary in insulin treated patients in order to avoid severe hyper- and hypoglycemia) constitutes an unacceptable reduction of individual freedom for many patients.

Furthermore incorrect measurements of the insulin amount in the syringe (20), reflux of insulin from the injection channel (20), subcutaneous degradation of injected insulin (3, 9), variations in insulin absorption rate between different areas of the body (4) and different tissue depths and variations in the motility of the stomach contribute to these difficulties. Also 1-3 injections a day are disturbing and may give rise to lipodystrophy, allergy and pain.

Abbreviation. IRI = immunoreactive insulin, MBG = mean blood glucose, MAGF = mean amplitude of glycemic excursions.

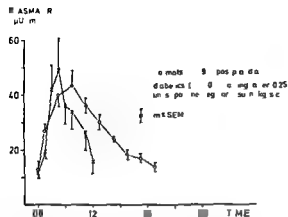


Fig. 1 Comparison of plasma IRI between 9 non-diabetics after breakfast and 10 fasting diabetics after subcutaneous injection of 0.25 U porcine regular insulin/kg body weight (mean \pm SEM).

In order to avoid hypoglycemia and unpredicted hyperglycemia, self monitoring of blood glucose has been introduced with good results in selected patient series (33). However, in a prospective study of consecutive insulin dependent diabetics with newly detected simplex retinopathy, Andersen et al. (2) did not achieve normalization of blood glucose profiles by this method. Therefore, and because of the severe drawback of the subcutaneous insulin therapy mentioned, *in vivo* insulin infusion systems are under evaluation in several places (1, 15, 16, 21). The aim of this paper is to compare the effects of two *in vivo* insulin infusion systems, the so-called closed and open loop system on glycemic regulation of insulin dependent diabetics in short term experiments.

SUBJECTS AND METHODS

Closed loop system. Twenty non-obese insulin dependent diabetics (3 females and 17 males, aged 18–46 years) who had had diabetes for 3–29 years, were examined under ward conditions after several days of attempt to achieve metabolic control with subcutaneous insulin therapy. Informed consent was obtained from all after careful explanation of the nature and purpose of the investigation. The clinical data appear from Table I.

Remaining endogenous insulin secretion was estimated by determination of human plasma C-peptide before and after an *in vivo* injection of 1.0 mg glucagon (Novo) by the method of Faber (12). C-peptide was not found in any of the 19 patients tested. They had been treated with highly purified porcine NPH insulin (Retard[®], Nordisk Insulinlab.) for several years, most of them with injections twice a day. The daily insulin dose varied between 0.40 and 1.28 U/kg body weight. None of the patients had acute illnesses.

The diet was strictly planned and served at the same time of the day in all experiments: breakfast at 8 a.m., lunch at 12 a.m., dinner at 6 p.m., snacks at 3 p.m. The composition and amount of food varied greatly according to the habits of each patient, generally about 30% of the daily amount of bread was at 8 a.m., 40% at noon, 10% at 3 p.m. and 20% at 6 p.m. The hot meal at 6 p.m. was served with or without potatoes but always with vegetables. A 30-min bicycle ride was performed by every patient at 10 a.m. and at 3 p.m.

Open loop system. Fifty-three insulin-dependent diabetics (19 females and 34 males, aged 14–56 years) with diabetes for 4–39 years and a weight of 74–134 kg were examined under the above conditions. These were comparable with those in the closed loop study regarding age, duration of diabetes, body weight, and complications (Table I).

C-peptide was found in one of the 35 cases who were tested for glucagon. In this case, only a very slight significant increase in C-peptide was demonstrated after glucagon injection (0.08 \rightarrow 0.14 pmol/ml) compared to 0.36 \rightarrow 1.78 pmol/ml in normals (12). The 35 patients in whom C-peptide was measured did not differ from others regarding age, duration of diabetes, body weight, insulin dose. The daily insulin dose varied between 1.10 U/kg body weight. The diet and physical exercise in the experiments were as described above.

Controls. Fifteen non-obese non-diabetic subjects (ideal body weight 97.9%, age 13–48 years, mean \pm SD) served as normal controls. Plasma immunoreactive insulin (IRI) fluctuations were measured in 9 of them.

Experimental design

When informed consent had been obtained, NPH treatment was discontinued and regular insulin administered subcutaneously 4 times daily, morning, noon, evening, and at bedtime, in order to maintain the patient on NPH insulin from the subcutaneous tissue before initiation of insulin infusion.

The patients were connected to an artificial closed loop Bostator[®] Miles or a portable infusion pump (open loop system) described elsewhere, at least 6 hours before the start of metabolic measurements.

The calibration of the artificial B-cell is described elsewhere (8) and algorithms were set to give at least amount of insulin which could normalize tolerance in juvenile diabetics (H.R. 0.01–0.02 U/kg body weight of glucose infused with Bostator was 6 hours). Basal insulin infusion was defined as the amount of insulin infused per kg per min between 8 a.m. and 5.00 a.m.

Insulin infusion with the portable infusion pump was preprogrammed according to the following guidelines: 8–10 a.m. 17% of the total daily amount of insulin infused; at 10–12 a.m. 8% of the total daily insulin amount; at 12–14 a.m. 17% at 14–16 a.m. 8% at 16–18 a.m. 17% at 18–20 a.m. 8% Between 22 and 08.00 a.m. of the total insulin amount was infused. Only small variations in the program were permitted. The insulin concentration in the solution in the infusion syringe varied however.

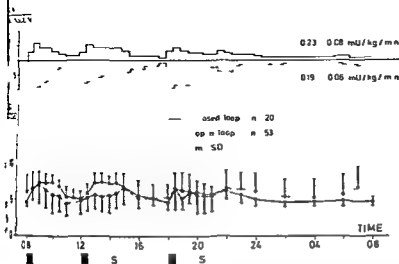


Fig. 2. Capillary blood glucose in 53 insulin-dependent diabetics treated with open loop system and venous blood glucose in 20 insulin-dependent diabetics treated with closed loop system ($\bar{V} \pm S.D.$) compared with capillary blood

glucose in 15 non-diabetics on diabetic diet ($\bar{V} \pm S.D.$) (shaded area). Insulin infusion rate in relation to basal insulin infusion is given on the top. Mean basal insulin infusion rate is given in the figure.

ten patients depending on the individual daily insulin requirement which was calculated on the basis of several patient visits and observations during hospitalization. Patients in the open loop group performed physical exercise of moderate severity on a bicycle ergometer (in heart rate about 40%) during fast and constant insulin infusion rate. Seventeen patients in the closed loop performed physical exercise of similar severity during prandial state three hours after a main meal and four after a snack.

Continuous blood glucose was measured continuously by the sensor in the closed loop system. In order to make comparisons with the open loop system, mean blood glucose (MBG) of the last 5 min before the time given in Fig. 2 was determined in each case. During experiments in the closed loop system, capillary blood was taken from the ear 30 times a day for whole blood glucose determina-

tion by the glucose oxidase method (34). Sampling hours are given in Fig. 2. Glucose in 24-hour urine samples was also determined by the glucose oxidase method. MBG ($\pm S.D.$) was calculated on the basis of all capillary blood glucose determinations per 24 hours. As a measure of blood glucose fluctuations in the individual patient, the mean amplitude of glycemic excursions (MAGE) was calculated by the method of Service et al. (28). Hypoglycemic reactions were recorded when the patient had appropriate symptoms and blood glucose levels lower than 2.5 mmol/l.

Plasma IRI was measured by the method of Heding (14) in 4 ketotic newly diagnosed diabetics in the open loop group during *in vivo* insulin infusion and in those 14 of the 20 closed loop patients who had no or only minimal amounts of insulin antibodies.

Statistical evaluation was carried out by Student's *t* test.

Table 1. Clinical data on the patients (mean $\pm S.D.$)

	Closed loop group (n=20)	Open loop group (n=53)
Age (y)	32.3 \pm 9.6	31.6 \pm 11.6
Duration of diabetes (y)	11.4 \pm 6.8	15.9 \pm 7.7
Ideal body weight	95.0 \pm 9.1	99.5 \pm 11.8
Insulin/kg b.w.t. subcutaneously (U)	0.73 \pm 0.22	0.67 \pm 0.24
Insulin ingested	2.117 \pm 3.01	1.889 \pm 3.03
Complications		
of patients with		
retinopathy	9	29
neuropathy	1	3
phropathy	6	25

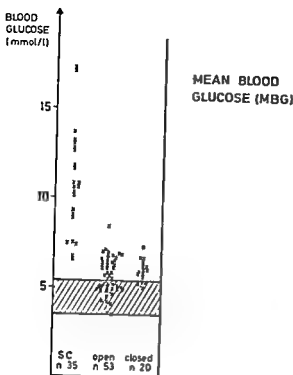


Fig 3 Individual MBG in insulin-dependent diabetics on subcutaneous insulin therapy (sc) treated with the open loop or the closed loop system. Shaded area = $\bar{M} \pm 2$ S.D. of 15 non-diabetics

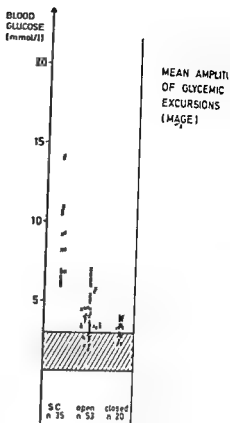


Fig 4 Individual MAGE in the same patients as in Fig 3. Shaded area = $\bar{M} \pm 2$ S.D. of 15 non-diabetics

RESULTS

Fig 2 shows the blood glucose variation during 24 hours of insulin infusion both with the closed loop and the open loop system. Most of the time MBG was within the normal range. In the daytime the open loop system gave lower blood glucose values

whereas they approached the physiological level with the closed loop system in the night. Fig 3 shows the individual MBG values during insulin infusion. MBG values for 35 of 53 patients underwent the same blood glucose measurement schedule as on the experimental day on the last

Table II Insulin infusion rate, insulin requirement, MBG, MAGE, glucosuria and hypoglycemia in insulin dependent diabetics treated with closed loop and open loop insulin infusion systems

	Control group (n=15)	Closed loop group (n=20)	Open loop group (n=53)	Significance of the difference closed/open loop
Basal insulin infusion rate (mU/kg/min)				
Maximal infusion rate (% of basal)		0.23 \pm 0.08	0.19 \pm 0.06	n.s.
Total insulin amount (U/24 h)		570	900	-
Subcutaneous		48.6 \pm 11.6	44.6 \pm 11.9	n.s.
I.v. infusion		47.9 \pm 12.2	44.4 \pm 12.8	n.s.
MBG (mmol/l)	4.4 \pm 0.5	6.0 \pm 0.6	5.8 \pm 1.0	n.s.
MAGE (mmol/l)	1.8 \pm 0.6	3.5 \pm 0.8	4.3 \pm 1.3	2p < 0.02
Glucosuria (g/24 h) (range)		0-5	0-16	n.s.
Hypoglycemia (n)		0	10	-

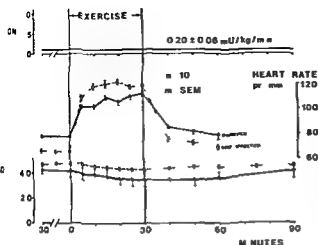


Fig. 5 Capillary blood glucose ($\bar{M} \pm \text{SEM}$) before, during and after physical exercise in 10 fasting non-insulin-dependent diabetics treated with the open loop system (\bullet) and 10 non-diabetics (\circ).

NPH treatment are given for comparison.

Apparently many of the patients infused with the open loop systems had values within the normal range, whereas only few infused with the closed loop systems were brought down to such levels. However, the difference in MBG between the two systems was not significant (Table II). The MAGE did not normalize in most patients with the closed or the open loop system (Fig. 3) but improved considerably compared to the patients' values when treated with subcutaneous insulin suspensions. It should be noted that MAGE does not normalize in patients on closed loop system. MAGEs were significantly higher in the open loop than in the closed loop group (Table II).

Where hypoglycaemic attacks were not seen in the open loop group. However, 10 of the 53 patients in the closed loop group reported hypoglycaemic feelings

together with capillary blood glucose values below 2.5 mmol/l. Some of the 10 patients in the closed loop group received glucose by the Medtronic in order to avoid hypoglycaemia, but the dose never exceeded 7 g/4 hours.

Urinary glucose excretion was 0–16 g/4 hours (mean 2.8) in patients in the open loop group and 0–5 g/4 hours (mean 1.0) in the closed loop group (Table II).

Capillary blood glucose concentration in the open loop group patients performing physical exercise during fast decreased only slightly in spite of a rather heavy workload with pulse increments of 40 b/min (Fig. 5). Postprandial exercise 3 hours after a main meal and one hour after snacks in the closed loop group led also to a small but significant decrease in venous blood glucose in 17 patients (Fig. 6) in spite of decreasing insulin infusion rates.

There was no difference in either group between

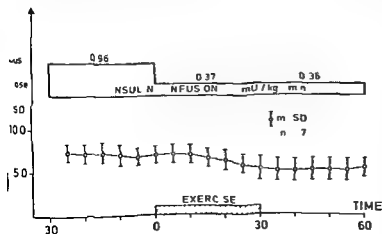


Fig. 6 Venous blood glucose ($\bar{M} \pm \text{SD}$) before, during and after physical exercise in 17 non-insulin-dependent diabetics 3 hours after a main meal and one hour after a snack. The patients were treated with a closed loop system in which the insulin infusion rate was dependent on blood glucose concentration. The amount of insulin infused 30 min before, during and 30 min after physical exercise was averaged. The effect of physical exercise on insulin requirements is obvious.

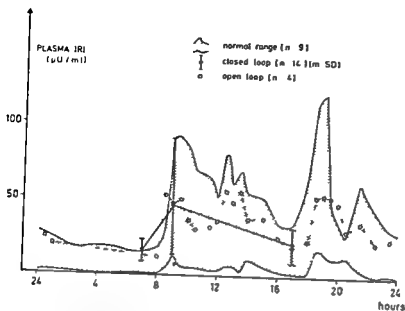


Fig. 7 Plasma IRI in the fast state (07.00 a.m.) postprandial (09.00 a.m.) and before dinner (05.00 p.m.) in 14 insulin-dependent diabetics (\bullet — \bullet) on closed loop treatment (\pm S.D.) and 4 patients with newly diagnosed insulin-dependent diabetes on open loop treatment (\circ — \circ) compared with the plasma IRI in 9 non-obese non-diabetic subjects on a diabetic diet (shaded area).

the total amount of insulin given on the last day of NPH treatment and on the infusion day (Table II). The basal insulin infusion rate (amount of insulin infused per kg/min between 1 and 5 a.m.) was the same in the two systems. The increase in infusion rate in per cent of the basal infusion was similar in the two systems (Fig. 2) and plasma insulin concentration was within the physiological range during infusion in both systems (Fig. 7).

DISCUSSION

The results demonstrated that the closed loop and open loop systems gave almost equally good blood glucose profiles in insulin dependent diabetics. During daytime blood glucose was lower in patients on treatment with open loop than closed loop system. In the night however it was the other way round. This is in accordance with the different infusion rates in the two systems: the open loop system infusing more insulin in connection with the main meals (Fig. 2). By changing the constants of the Biostat[®] better results could possibly be achieved but only by infusion of larger amounts of insulin. A second possibility that might result in normalization of MBG and MAGE without increment of the amount of insulin infused would be to preprogram the closed loop system to increase the insulin infusion at the very beginning of meals. The standard deviation of MBG is lower in patients treated with the closed loop system and also the MAGE of closed loop system patients seems to be more physiological than in patients treated with the

open loop system. The unphysiologically high MAGE in patients treated with the closed loop system might reflect the instability of the glucose oxidase membrane and problems with the sampling catheter.

Physical exercise during fasting conditions with low constant insulin infusion resulted in a slight decrease in the capillary blood glucose concentration. Postprandial exercise performed while the insulin infusion is high may however lead to a considerable decrease in blood glucose concentrations if the infusion rate of insulin is not previously decreased as it was in our experiments.

The infused amount of insulin did not differ from that given subcutaneously. This does not rule out the possibility of slight subcutaneous insulin degradation (9) since the subcutaneously administered insulin dose should eventually have been a little higher in order to achieve better metabolic regulation.

Plasma insulin concentrations during infusion were within the normal range. This is in accordance with findings by others (27–29). Thus it does not seem necessary to induce peripheral hyperinsulinemia during *in vivo* insulin infusion in order to normalize blood glucose in insulin-dependent diabetics without endogenous insulin secretion as claimed by Botz et al. (7).

Long term infusion of insulin has been demonstrated to normalize not only plasma blood glucose concentration but also other metabolic hormones (22–23, 31–32) often found in physiological amounts in insulin treated diabetic

subcutaneous insulin injection therapy. Long-term infusion is however not realistic since application of catheters in the subclavian vein for long-term use is reported to have a frequency of complications between 0.5 and 8% (25). Sepsis arising from these catheters has been found in 0.8–1.6 years of catheter application (19). Furthermore, the frequency of thromboses and embolism cannot be neglected. Intraperitoneal infusion of insulin is therefore under consideration (24–26).

Subcutaneous insulin infusions have been investigated by several groups (23–31, 32). However, the effects of blood glucose regulation in insulin-dependent diabetics without endogenous insulin secretion are not as good as those of i.v. infusions, probably due to the reproducibility of the absorption of insulin from the subcutaneous tissue is not good enough and because it takes too much time before a maximum insulin peak concentration is achieved after a square wave pulse from the infusion system (30). Even implantable closed loop systems can probably not be constructed in the near future, mainly due to difficulties with the glucose sensor, miniaturized pumps for implantation applicable for long-term loop systems are now being constructed. Such pumps should be constructed with at least programs of infusion profiles which could be controlled by the patient himself since only one program turned out to be insufficient for insulin-dependent diabetics on totally free diet (10). Another difficulty might be the insulin concentration in the reservoir in the reservoir of such pumps. In our study, a solution of highly purified porcine insulin 500 U/ml was stable at 37°C for several hours. A precipitation of insulin might however occur during agitation of such solution. Studies are in progress to solve these problems.

CONCLUSION

Under standard conditions (planned meals and exercise) closed loop as well as open loop systems can keep blood glucose and plasma insulin near the normal range in insulin-dependent diabetics without endogenous insulin secretion. Blood glucose control with the closed loop system is however not as good as that with the open loop system, since standard deviations of MBG and MAGE are smaller with the former than with the latter system. The risk of developing severe hypoglycemia during

exercise seems to be small, but it has to be found out whether blood glucose fluctuations resulting from improvisations regarding time and quantity of meals during daily life can be regulated by open loop infusion pumps.

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Studies on Hemopoietic L lasia (the Preleukemic Syndrome)

Clinical Course and Prognostic Factors in 42 Patients

Listed Bone Marrow

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ABSTRACT The clinical course of 42 patients with myeloid dysplasia diagnosed during 1974-77 has been studied. The clinical, laboratory and morphological findings at presentation were investigated for prognostic significance. At the time of completion of the study (Aug. 1979), 38% had developed acute leukemia (median survival after diagnosis 5 months); 15% had died of complications (bleeding and infection) due to severe cytopenia (median survival 6 months). An overall malignant course was observed in patients with low platelet count ($<80 \times 10^9/l$), bleeding, and a severe myeloid maturation defect in the bone marrow ($>30\%$ myeloblasts and promyelocytes). Increased serum vitamin B₁₂ and presence of blasts, promyelocytes and abnormal mononuclear cells in the peripheral blood indicated a leukemia.

Parameters assumed to be of prognostic value were compared and related to the time of survival in patients who developed leukemia and patients who died of cytopenia.

PATIENTS AND METHODS

Forty-two patients (25 men and 17 women, mean age 69 years) who had presented with dysplastic bone marrow at our department in 1974-77 were studied. The definition of HD in this investigation included patients with both normal and increased numbers of myeloblasts and promyelocytes in the bone marrow (Vitter et al. 1967, Type I and Type III refractory anemia; Dreyfus et al. 1970, Refractory anemia with excess of myeloblasts). The details of definition and the patients examined have been described elsewhere (9).

Diagnostic examination

The clinical and laboratory parameters examined for prognostic significance were: a history of hemorrhagic episodes and/or recurrent infections; hemoglobin (Hb) $<80 g/l$; white blood cell count (WBC) $<2.5 \times 10^9/l$; platelet count $<80 \times 10^9/l$; mean corpuscular volume (MCV) increased; serum vitamin B₁₂, serum iron, reticulocyte count, lactate dehydrogenase (LD) and morphological abnormalities in the blood and bone marrow. For evaluation of the myeloid maturation defect in the bone marrow, a 4 graded scale was used referring to the relative number of myeloblasts and promyelocytes with the ranges 8-19, 20-29, 30-39 and 40-50%.

The parameters under consideration were: 1) examined in (a) patients who developed acute leukemia and (b) patients who died of HD without leukemia; 2) Related to a survival time less than 3 months (b) 3-12 months (c) more than 12 months after diagnosis.

The statistical methods used were Fisher's exact test and the fourfold table test.

Abbreviations: AML = acute myeloid leukemia; AMML = acute myelomonocytic leukemia; EL = erythroleukemia; Hb = hemoglobin; HD = hemopoietic dysplasia; LD = lactate dehydrogenase; MCV = mean corpuscular volume; WBC = white blood cell.

myeloid dysplasia, preleukemia, clinical course and prognostic factors.

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Myeloid dysplasia (HD, the preleukemic syndrome) is a disorder with a high mortality rate (1, 3) that terminates frequently in acute myeloid leukemia (AML). A number of factors believed to be of prognostic significance have been described in patients. Severe anemia (8, 13), reduced platelet count (8, 17, 20), chromosomal abnormalities (13, 14, 17), absence of typical ringed neutrophils (6) or increasing numbers of myeloblasts in the bone marrow (7, 8, 12) have been related to indicate an increased risk of acute leukemia transformation. However, many patients develop leukemia but succumb from effects of severe cytopenia and some appear to have a benign course (11, 18) over several years. The clinical, laboratory and morphological findings in patients with dysplastic bone marrow have been examined and are presented in this study.

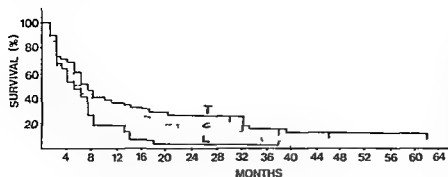


Fig 1 Survival from time of diagnosis. T = total series. C = patients with cytopenic complications. L = patients who developed acute leukemia. Short vertical lines indicate patients still alive.

RESULTS

When the clinical and morphological examination had been completed (Dec 1977) 31 patients had died. Thirteen deaths were due to acute leukemia (7 AML, 4 acute myelomonocytic leukemia (AMML), 2 erythroleukemia (EL)) and 18 due to complications of cytopenia (bleeding, infection). One year later, 5 more patients had died (3 of AML and one each of severe cytopenia and myocardial infarction). Of the 17 patients (40%) who until Aug 1979 had developed leukemia, only one (a male aged 36) entered remission after chemotherapy and he is still alive. The median survival, estimated from the diagnosis of HD, was 7 months (mean 23), 5 months (mean 14) for the patients who developed acute leukemia and 6 months (mean 32) for those who died in HD (Table I). Five patients with a stable dysplasia are still alive 30–62 months after diagnosis (Fig 1).

Prognostic factors

Parameters of significance in varying prognostic categories (Table II) were: 1) Patients who developed acute leukemia differed from those who died of HD in (a) number of patients with increased serum vitamin B₁₂ (10/16 vs 1/14, $p < 0.01$)

and (b) number of patients with myeloblasts, myelocytes and abnormal monocytoïd cells in blood (6/17 vs 1/19, $p < 0.05$). 2) Patients who lived less than 3 months after presentation differed from those who survived 3–12 months in number of patients with a history of hemorrhagic episodes (10/12 vs 5/15, $p < 0.05$) and (b) number of patients with $\geq 30\%$ myeloblasts and promyelocytes in the bone marrow (11/12 vs 4/15, $p < 0.05$).

Patients who survived less than 12 months differed from those who survived longer (including 5 dysplastic patients still alive) in (a) number of patients with bleedings (15/27 vs 2/13, $p < 0.05$) and (b) number of patients with a platelet count $< 50 \times 10^9/l$ (14/27 vs 2/13, $p < 0.05$) and (c) number of patients with $\geq 30\%$ myeloblasts and promyelocytes in bone marrow (15/27 vs 0/13, $p < 0.001$).

Other clinical and laboratory parameters examined (a history of recurrent infections, $E. coli$, WBC $< 2.5 \times 10^9/l$, increased MCV, serum reticulocyte count and LD) or other blood cell features (severe red cell abnormalities, presence of immature myeloid or monocytoïd cells) and marrow abnormalities (dyserythropoietic features, myeloid cellular abnormalities, megakaryocyte maturation defects) did not differ significantly.

Table I Clinical course of the 42 patients

Present state	N	%	Clinical outcome	N	%	Final complications		Median survival (mo) ^a
						Bleedings	Infections	
Dead	36	86	AML/AMML/EL	16	38	4	8	5 (14–3)
			HD	19	45	9	13	6 (3–4.5)
			Myocardial infarction	1				
Alive	6	14	AML in remission	1				38
			HD	5				38 (41–30.4)

^aMean and range given in parentheses.

Table 1 Prognostic factors

	Development of leukemia (n=17)	Dead in dysplasia (n=19)	Survival (mo) *			
			<3	3-12	>12	>12
raised serum B ₁₂	10/16	1/14*	5/8	5/15	10/23	1/10
myeloblasts, promyelocytes and myeloid cells in the blood	6/17*	1/19	3/12	3/15	6/27	2/13
platelet count <60 × 10 ⁹ /l	10/17	6/19	10/12*	5/15*	15/27	2/13
myeloid myeloblasts and promyelocytes ≥ 30%	6/17	10/19	9/12	5/15	14/27*	2/13*
	8/17	6/19	11/12 *	4/15**	15/27 *	0/13*

* Examined in 33 patients only

** 10 patients (one who died of myocardial infarction and another with AML in remission) were excluded from the analysis

*** p < 0.001

ed in the different patient groups or when related to the time of survival

DISCUSSION

Hemopoietic dysplasia transforms into acute myeloid leukemia in about 3/4 of patients within 2 years after diagnosis (11). However, as much as 50% (2/12) have been reported to die from septic complications, a finding largely in agreement with this study. Thus, HD has a high overall mortality rate which has so far reached near 90% in the present examination.

The median survival time of 7 months in this study differs from that of 26 months reported by Appel et al. (7) and there is a considerable variation in the survival of patients with HD. In the present study one patient died only one month after diagnosis and another is still alive and well after 5 years. Extremely long and benign courses up to 17 years have been reported (10, 14, 15). However, such patients may still have an increased risk of a fatal outcome with or without developing leukemia (1) and should be controlled as regularly as those with a more rapid course.

Some parameters of possible prognostic significance have been demonstrated (Table II): a few of which (reduced platelet count and myeloid bone marrow maturation defect) are in accordance with earlier studies. However, it was somewhat unexpected to find an increased serum vitamin B₁₂ level frequently in patients who developed leukemia than in those who did not. It has been suggested (14) that an elevated serum B₁₂ might be related to increased promyelocytes in the bone marrow

(transcobalamin I is believed to be a product of the granules of promyelocytes) and serum B₁₂ has been reported to be elevated in some patients with acute leukemia (16). The increased vitamin B₁₂ found in some HD patients might reflect the myeloid maturation defect at a stage when many of the myeloid cells are promyelocytes.

In this study patients with low platelet count, bleedings and a severe maturation defect (≥ 30% myeloblasts and promyelocytes) in the bone marrow appeared to run a malignant course with short survival. Thus, the evaluation of such simple clinical parameters can be of some prognostic value and can also be used in hospitals without specialized hematological units. However, the prediction of the clinical outcome from laboratory and morphological data might not be quite reliable, but the use of modern methods such as in vitro culture of granulopoietic stem cells (4, 19) has added more reliability to the prediction of the clinical course in HD. In vitro culture studies in combination with morphological examination might increase the possibility to predict the course. Such studies are in progress.

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Sinus Node Dysfunction in Acute Myocardial Infarction

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ABSTRACT In a retrospective study of patients admitted for acute myocardial infarction (AMI) during 1977, sinus node dysfunction (SND) was detected in 104%. Twelve had persistent sinus bradycardia, sinoatrial block or sinus arrest and six bradycardia syndrome. Symptoms occurred in 17 patients, of whom four required temporary pacing for periods of 1-3 weeks. A permanent cardiac pacemaker was implanted in three patients with bradycardia.

Three patients died during the primary attack and six during the observation period. Following a mean observation period of 34 months, continuous signs of SND in 11 out of 19 patients. The arrhythmia caused symptoms in five patients, two of whom had a cardiac pacemaker and received medical treatment. It is concluded that SND appearing during an AMI persists in a high proportion of these patients.

KEY WORDS: sinus node dysfunction, acute myocardial infarction, cardiac pacemaker treatment.
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During the last decade, sinus node dysfunction (SND) has been increasingly recognized as a cause of cardiac arrhythmia. The various manifestations of SND are known to occur during the acute phase of myocardial infarction (1, 2). Parameswaran et al. (3) suggested a different persistence of the various types of SND, particularly the bradycardia syndrome, in patients with acute myocardial infarction (AMI). The aim of the present study was to find out whether other types of SND carry any prognostic significance.

PATIENTS AND METHODS

Records of all patients with AMI admitted to the CCU at the University Hospital during a period of approximately six years (April 1, 1972-Dec 31, 1977) were reviewed. A definite diagnosis of AMI according to the criteria was made in 2789 patients, 71.4% of them men. The total hospital mortality was 24.7%. In all

patients the following information was available: daily electrocardiogram (ECG), frequent registration of clinical data (blood pressure, pulse rate, attacks of chest pain, administration of analgesic and antiarrhythmic drugs). The cardiac rhythm was monitored continuously for an average of 5-6 days. Rhythm strips were recorded automatically in case of bradycardia or tachycardia. In addition, any changes in cardiac rhythm observed by the technicians could be documented in ECG tracings using a tape recorder with delay.

The following ECG criteria for SND were used: 1) Persistent sinus bradycardia of less than 50 beats/min during at least 16 hours; 2) Sinus rhythm with episodes of sinoatrial block or sinus arrest; 3) Bradycardia syndrome, i.e. characteristics as above combined with attacks of tachycardias of supraventricular origin.

Patients with previously known SND and those on digoxin, β -blocking agents or other antiarrhythmic drugs were excluded. Patients in whom SND occurred during cardiogenic shock or cardiac resuscitation or was suspected to have been provoked by pain, morphine, analgesics or vomiting were also excluded.

A follow-up including patient interview, clinical examination, X-ray of the heart and lungs and one minute 12-lead ECG was carried out in Sept.-Oct. 1978. Available additional information (records from hospital admission, death certificates, etc.) was also taken into account.

RESULTS

Twenty-nine patients (5 women and 24 men) met the criteria for SND. Some of the clinical data are given in Table 1. The mean age was 66 years (range 47-83). Previous heart disease was noted in 13 patients (ischaemic in 11, rheumatic in one, hypertensive in one). In accordance with the ECG criteria mentioned, the cases were divided into three groups.

Group 1: sinus bradycardia (cases 1-12). In five patients the bradycardia was accompanied by minor symptoms (mild hypotension, dizziness), but syncope occurred in one patient with ventricular fibrillation. The patients were treated with atropine.

Abbreviations: SND = sinus node dysfunction; AMI = acute myocardial infarction; ECG = electrocardiogram.

Table I Clinical data on 29 patients with AMI and SND

I = inferior A = anterior SB = sinus bradycardia SAB = sinoatrial block SA = sinus arrest AF = atrial fibrillation VF = ventricular fibrillation VPB = ventricular premature beats AVB = atrioventricular block VT = ventricular tachycardia SVT = supraventricular tachycardia (+A) = autopsy performed

Case no	Sex	Age (y)	Location of AMI	Type of SND	Syncope	Associated arrhythmias	Pacemaker treatment		Follow up		
							Temporary (d)	Permanent	Observation time (mo)	Type of SND	Causes of death
1	♂	47	I	SB		VPB			42		
2	♂	70	A	SB		AVB 1			55		
3	♂	58	I	SB		VPB			41	SB	
4	♂	52	I	SB					12	SB	
5	♂	67	I	SB		VPB			60		
6	♂	57	A	SB					37	SAB	
7	♂	54	A	SB					16		
8	♂	68	?	SB					17	SB	
9	♂	67	A	SB	+	VF			19	SB	
10	♂	64	I	SB		VPB			16 (†)		Probable MI
11	♂	77	A	SB		VPB			25 (†)		Pulmonary oedema
12	♀	69	A	SB					7 (†)		Probable MI
13	♀	71	A	SAB					51	SAB	
14	♂	66	I	SA	+	AVB 3	16		25		
15	♂	63	I	SA SB			10		58	AF	
16	♂	73	I	SAB					27		
17	♂	66	I	SAB SB			4		54	SB	
18	♂	76	A	SA	+	VF	4		25		
19	♀	77	I	SAB					28 (†)		Respiratory insufficiency
20	♂	59	I	SAB		VPB			-		
21	♂	70	I	SA SB	+	AVB 2	4 (†)				
22	♀	83	I	SA			2 (†)				
23	♂	77	?	SAB					44 (†)		Carcinoma of pancreas (+)
24	♂	72	?	SAB AF			10	+	20	Pace rhythm	
25	♀	55	I	SA AF	+	AVB 2	14		18	Paroxysmal AF	
26	♂	64	I	SB AF		VT	3		24		
27	♂	73	I	SA AF	+		3		18 (†)		Carcinoma of colon (+A)
28	♂	59	A	SA SVT	+	VF	7	+			
29	♂	67	I	SA AF		VPB	23	+	60	AF	

0.5–1.0 mg i.v. resulting in transient increase in heart rate in five. Other arrhythmias and conduction disturbances were noted in seven patients (Table I). The sinus bradycardia lasted for only 1–3 days in most of the patients, yet in two patients throughout their stay in hospital.

Group 2 sinoatrial block/sinus arrest (cases 13–23). This group included 11 patients. SND induced symptoms in six patients, three of whom had one or more syncope due to sinus arrest. Significant associated arrhythmias appear from Table I. Six patients required temporary pacing, two of these died

in cardiogenic shock and four were paced 1–5 days. Signs of SND were present during the hospital stay in three patients and during 1–10 days in the others.

Group 3 brady tachy syndrome (cases 1–12). All six patients in this group experienced symptoms (syncope, profound hypotension) requiring temporary pacing. Treatment with isoprenaline attempted in four patients failed to maintain adequate heart rate. Temporary pacing was continued for 3–23 days. In three patients it was terminated within two weeks, whereas a permanent

II Follow up of 19 patients with SND during

	Group 1	Group 2	Group 3
no. of pats	12	11	6
deaths	3	4	2
pats followed	9	6	4
observation time			
	32	40	30
pats with signs			
D	5	3	3
ent of pats			
ND			
	5	2	0
ad	0	1	1
anent pacemaker	0	0	2

pacemaker was implanted in three. One patient died from ventricular fibrillation on the day of implantation. In the entire patient group inferior infarction was frequent (17 patients). However the ratio of inferior to anterior infarction was almost 1 in group 1, a marked predominance of inferior infarction is noted in group 2 (8/2) and group 3 (4/1). In 11 of the 19 patients SND was observed within 48 hours of the onset of AMI.

UP

One patient was lost for follow up, three died during the first hospital admission and six during the observation period. The remaining 19 patients were followed after a mean observation period of 34 days (range 12-60).

The mortality was 20-25% in each of the three groups (Table II). Extracardiac disease was the cause of death in the three autopsy cases (Table I). Death certificates of the three cases without autopsy indicated heart disease as the cause of death. No positive indications of systemic embolism or sudden cardiac arrest was given but unfortunately the information about these three cases is not enough to allow conclusions regarding the cause of death.

SND could be demonstrated by ECG tracings in 11 patients. In addition two patients had a permanent pacemaker. Sinus bradycardia (rate 45-50) was present in five patients and sinoatrial block in two patients. Originally in group 2 (case 15) had atrial flutter with a slow ventricular rate and was treated with isoprenaline. Another patient (case 25) was treated with quinidine because of

paroxysmal atrial fibrillation. Apart from these two patients only one, namely a patient with sinoatrial block, had complaints that could be related to SND. The symptoms were insignificant (slight dizziness and palpitation) and the patient managed without any treatment.

DISCUSSION

The frequency of SND in AMI patients has been reported to vary from 4 to 5% (1, 2, 3). This figure is of course dependent on the inclusion and exclusion criteria chosen. We found a frequency of 1.04%. The retrospective collection of the patient series may have caused some drop-outs, but the rather low figure is ascribed particularly to the fact that many cases were excluded because of drug treatment. In accordance with Parameswaran et al (1) we have found a preponderance of inferior infarction and onset of SND within the first 24 hours after onset of an AMI in most of our patients. Also the finding that treatment with isoprenaline was ineffective agrees with the results of Parameswaran et al but not with those of Rokseth and Hatle (2). The latter authors found that temporary pacing was necessary in a few cases only. The former authors have pointed out that the treatment of patients with brady-tachy syndrome presents particular problems. They found that five out of six patients required continuous antiarrhythmic therapy, four of them in addition to permanent pacing. Similarly we found that three of the four survivors in group 3 needed treatment with antiarrhythmic drugs and two permanent pacing as well.

Parameswaran et al presented follow up information covering periods of 10-30 months. The main part of this information was provided by general practitioners. Among their 17 patients they found continuous signs of SND in six, one of whom belonged to the subgroup with sinus bradycardia and/or sinoatrial block. At follow up we found signs of SND in 11 of 19 patients. Only five patients showed symptoms which could be related to the arrhythmia.

The detection of SND at follow up in nearly 60% of our patients in a one minute ECG is somewhat surprising. As SND is often intermittently present this figure might be considered a minimum. Patients with previously known SND were excluded from the study. As regards patients with brady-tachy syndrome there seems to be no doubt that the

arrhythmia appeared for the first time during the AMI. In cases of sinus bradycardia/sinoatrial block the possibility of pre-existing (asymptomatic) SND cannot be completely ruled out. This might be an explanation of the demonstration of SND in eight out of 15 patients at follow up. However, in a study of 128 patients with symptomatic SND (4) only two experienced an AMI during a mean observation period of three years. Assuming a similar incidence of AMI in patients with asymptomatic SND, it can be calculated that this population should be very large to contribute significantly to the present material. Although the ratio asymptomatic/symptomatic cases of SND in the general population is unknown, we assume that SND made its debut during AMI in patients in groups 1 and 2 as was the case regarding patients in group 3.

The reported observation seems to indicate that SND appearing in AMI persists in a considerable number of patients. In brady-tachy syndrome treatment is most often necessary. Symptoms ac-

companying other types of SND seem to be and inconstant and thus only occasionally treatment.

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Blood Pressure Control in a Middle-Aged Male Population

*A 6-9 Year Follow up with Special Reference to the Problem
of Non Responders*

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ABSTRACT A health examination survey comprising 2322 men born in 1920-24 was carried out in 1970-73 at the University Hospital Uppsala. Selected subjects with a diastolic BP ≥ 105 mmHg, those already on treatment for hypertension, and those considered hypertensives. If a BP elevation was found at a second screening and the patient had established contact with a physician, he was offered treatment. Thus 83 men have been followed for at least 6 years. The overall reduction in SBP after 6 years was 29.0 mmHg and in DBP 19.6 mmHg. Six men have died, four of them from myocardial infarction, five have dropped out for other reasons. The percentage of non responders (DBP ≥ 105 mmHg) 1, 2, 3, 4 and 5 years ± 3 months after the start of treatment was high, about 20% on the average. On the other hand, when the mean BP of the non responders at all visits to the hypertension clinic was compared with their pretreatment values, a reduction of 29.3 mmHg in SBP and 17.0 mmHg in DBP was achieved. The results with regard to compliance and BP reduction are encouraging and would seem to justify more widespread screening and treatment.

Keywords: blood pressure control; non responders; middle aged men.

Acta Med Scand 208 467 1980

Community control of hypertension has been the subject of a special WHO report (10). In this report it is considered inadvisable to start widespread population screening before successful pilot programmes have been carried out. The reason given is the necessity for answering certain preliminary questions, for example: Is it possible to normalize blood pressure (BP) and to keep it normal in all people? Can symptom free hypertensives

continue with life long treatment? What will be the psychological response to long term therapy in people without symptoms from their disorder?

This article deals with some of these problems on the basis of a programme aimed at bringing elevated BP under control in middle aged men.

STUDY POPULATION

All men living in the urban district of Uppsala and born in 1920-24 were invited to undergo a health examination between 1970 and 1973. A total of 2322 men were examined, giving a participation rate of 83.9% (3).

Untreated subjects with a supine diastolic BP (DBP) ≥ 105 mmHg and those already on treatment for hypertension were considered hypertensives. There were 174 men who met these criteria. If BP elevation i.e. supine DBP ≥ 105 mmHg was verified at a second screening examination at a special hypertension clinic (HT clinic) they were offered treatment at this clinic, excluding 91 men with previously established contact with a physician. Thus 83 men remained. They have been continuously followed up for up to 9 years. The results are given in this paper.

METHODS

The BP, in the supine position after 10 min rest and in the erect position after 2 min of standing are recorded by a nurse at the HT clinic. The DBP is read at disappearance of all sounds (Korotkoff phase V) and recorded to the nearest 5 mmHg mark.

If possible the subjects were seen monthly until a sufficient BP reduction was achieved and thereafter at longer intervals, always by the same physician (one of us). When the BP was well under control they were seen twice a year.

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Abbreviations: BP = blood pressure; SBP = systolic BP; DBP = diastolic BP; HT clinic = hypertension clinic.

Table 1 BPs (mmHg) at the first screening and at the initial examination at the HT clinic (n=83) and annual progress during the following 6 years (n=72) (mean \pm S.D.)

	Supine		Standing	
	SBP	DBP	SBP	DBP
Screening	177.4 \pm 19.9	113.2 \pm 8.0	174.1 \pm 21.1	117.6 \pm 9.4
Initial examination	177.5 \pm 24.2	113.3 \pm 11.2	174.8 \pm 24.6	119.4 \pm 11.3
After				
1 y	150.9 \pm 19.9	97.7 \pm 8.4	148.1 \pm 18.7	103.6 \pm 8.0
2 y	147.7 \pm 17.5	96.4 \pm 9.2	145.3 \pm 17.7	103.2 \pm 7.1
3 y	145.5 \pm 19.8	94.3 \pm 10.1	144.7 \pm 20.4	101.2 \pm 9.5
4 y	142.9 \pm 16.1	94.0 \pm 9.2	141.7 \pm 15.6	100.1 \pm 8.8
5 y	147.1 \pm 17.3	94.0 \pm 9.3	149.3 \pm 20.0	101.1 \pm 10.1
6 y	148.5 \pm 19.7	93.7 \pm 9.2	146.9 \pm 19.4	100.6 \pm 9.7

Propranolol (Inderal[®]) was given as the first drug to the previously untreated subjects. The next step, if necessary, was to add hydralazine (Apresolin[®]) and the following if the BP was still not satisfactory to add diuretics.

Conventional statistical methods were used in the analyses.

RESULTS AND COMMENTS

Blood pressure reduction

Table 1 gives the BPs at the first two screening examinations and the annual values for the remaining follow-up period. All have now passed the 6-year observation mark. The DBP decreased gradually as did the systolic BP (SBP) during the first years whereafter a slight increase was seen. After 6 years the average reduction of the supine SBP

was 29.0 mmHg and of DBP 19.6 mmHg for whole group.

The mean of all BPs during the 1-5 years after commencement of therapy was calculated for each individual. The relation between this value and the pretreatment value is presented in Fig. 1 and the difference is the mean reduction achieved for an individual subject (Fig. 2). The reason for excluding the BPs in the first year that treatment was instituted gradually during year

At each annual follow-up—1, 2, 3, 4 and 5— \pm 3 months after commencement of therapy, 39 had not normalized (DBP < 105 mmHg) as a certain number of men (Table II). These 39 non-repon-

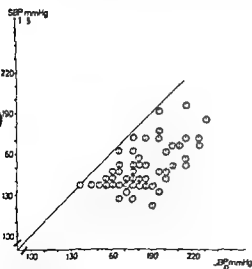
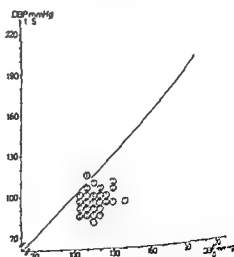
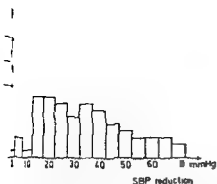


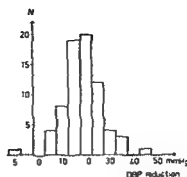
Fig. 1 BP development over the years. SBP, DBP₀ = average of the two initial pressures; SBP, DBP₁₋₅ = average of the pressures at all visits to the HT clinic in the



follow-up period 1-5 years after institution of treatment. Figures in the circles indicate number of men with particular BP initially and during follow-up.



2. Number of individuals with a particular SBP and DBP reduction. The reduction comprises the difference between the average of the two initial SBPs and DBPs



and the average of all SBPs and DBPs at the HT clinic 1-5 years after initiation of therapy

be referred to below as the failure group. As in Table II a substantial percentage of the series was in this group. However, it is noteworthy that the composition of the failure group is not identical from year to year. On the other hand, when the mean of all BPs during the follow-up period was calculated for all individuals in the failure group ($n=39$), it was found that the mean was on the average 21.9 mmHg below and mean DBP 12.9 mmHg below the pretreatment level of the total group ($n=83$). In the failure group the mean SBP was 29.3 mmHg lower and mean DBP 17.0 mmHg lower in the follow-up period than at the screening.

The failure group is analysed further in Table III. Details of 10 men appearing more than twice in the 5-year period are given in Table IV. A considerable average BP reduction occurred even in this group. Several of those who did not reach a level below 105 mmHg had had high initial BPs.

Medication adherence

There were 5 drop-outs during the 6-9 year follow-up period after 4, 5, 16, 34 and 36 months. Two of

them had alcohol problems, one preferred to visit his company doctor and two refused.

Mortality and morbidity

Six men died: four from myocardial infarction, one from injuries after an accident, and one committed suicide. This man was depressed after an invalidating accident and there was no reason to suspect a drug-induced depression. No episodes of cerebrovascular lesion occurred during the follow-up period.

Antihypertensive medication

Table V shows the type of drugs used at present. The single drug medication most commonly given is β -adrenoceptor blocking agents. Initially, one purpose of the study was to evaluate the possibility of using β -blockers to normalize the BP in a preventive trial like the present one. Later the trend has rather been to add other drugs than to increase the dose of β -blockers to a high level. The number of men receiving combinations of β -blocking agents and hydralazine and/or diuretics has therefore increased with time.

Table II. Men with DBP ≥ 105 mmHg at annual follow-ups

Follow up time	No. of non responders (%)
1-5	28.4
6-10	23.2
11-15	21.3
16-20	14.5
21-25	20.3

Table III. Number of men appearing once or more in the failure group at 5 annual follow-ups

	No. of non responders
Once	21
Twice	8
Three times	5
Four times	4
Five times	1

Table IV Individual mean BPs at the two screening examinations and the mean of all BPs in or the same subject in the failure group at 3-5 annual visits during the 5 year observation period. Figures in parentheses indicate number of BP measurements.

	Mean BP (mmHg)	
	At screening (×2)	Within 1-5 years
Three times	212 5/127 5 ^a 185/110 175/110 165/112 5 177 5/117 5	192 2±22 7/108 4±10 5 (75) 167 8±12 1/101 5± 8 0 (20) 155 5± 8 1/100 8± 5 3 (19) 147 3±11 9/102 3± 7 8 (15) 155 6±12 9/102 5±11 0 (8)
Four times	192 5/112 5 195/127 5 ^b 202 5/127 5 195/107 5 ^{a,c}	166 5± 9 0/107 3± 8 7 (24) 168 2±11 3/106 0± 8 8 (22) 150 0±14 7/107 8± 8 6 (18) 190 0±26 0/115 0±13 0 (15)
Five times	222 5/130	175 3×12 3/106 8± 5 7 (20)

^a Previously treated ^b Renovascular hypertension ^c Malignant hypertension and poor compliance in therapy

In two cases it was necessary to withdraw the β blocking drug because of adverse effects namely bradycardia in one and weight gain in the other. In another two cases the β blocker was withdrawn more or less for psychological reasons. Another change during the present follow up has been the introduction of selective β blockers (atenolol and metoprolol). Thus in 11 subjects unselective β blockers were replaced by selective ones due to possible side effects suspected bronchial obstruction in five CNS related side effects in four and impotence in two cases. Two further subjects in whom unselective β blockers had been considered contraindicated were given selective ones. One subject developed symptoms of systemic lupus

erythematosus with a positive antinuclear factor while on hydralazine. One man developed renal glycosuria while on a thiazide. At the screening had a fairly low K value 1.05 in an iv glucose tolerance test.

DISCUSSION

This paper deals with some problems encountered in an ongoing preventive trial aimed at curbing hypertension in middle aged men. All physicians working in this field are well aware of the problems accompanied by preventive treatment. Most of the participants are asymptomatic individuals with their own opinions sometimes definite on the necessity of instituting therapy or they may be very reluctant to accept care as suggested. Another fact to be considered in handling of these subjects is the importance of arranging their contacts with a physician without disturbing too much their ordinary daily life.

The BP reductions over the years must be considered acceptable in view of the special problems mentioned above. The number of non responders varies considerably with the definition used. Using the definition supine DBP ≥ 105 mmHg, the number is high. During the first 5 years about 10% of the subjects fulfilled this criterion each year. If a non responder is defined as a person showing a decrease in mean arterial pressure of less than 10% only two subjects who were examples of poor compliance would meet this criterion. The responders as a group also showed a substantial

Table V Type of present medication (6-9 years after the start of therapy)

	Per cent of all subjects (n=72)
Single drugs	
Adrenergic β -blocking agents	11.1
Diuretics	2.8
Combinations	
Adrenergic β blocking agents + hydralazine	29.2
Adrenergic β blocking agents + diuretics	26.4
Adrenergic β blocking agents + hydralazine + diuretics	19.4
Other drugs or combinations	11.1

tion of their BPs when measured over the 6-year period (Tables III and IV). As pointed out by others (9) a partial reduction of BP should also

be made. Very few comparisons with the literature can be made as only short follow ups in similar preventive trials have previously been presented (1-7). The former two studies give results for up to two years and the latter for up to one year. The periods that can be compared the BP reduction is almost identical.

Drop-outs during the 6-9 year period were few, one of the long follow up and relatively fewer in the other reported series (1-7-8) though the latter study population is smaller. The good patient adherence must mean that middle aged men are concerned about their health. It should be stressed that this group of subjects has been very well followed in one respect. All of them have met their doctor over the years which has been a repeatedly repeated reason given by the subjects for their good compliance. Other reasons might be the easy accessibility of the treatment centre and the fact that the HT clinic is open in the evening. Although this is only a small scale experiment in preventive medicine we feel encouraged by the results and the good patient compliance to use the more widespread community screening and treatment programmes. The outcome of such community efforts is however dependent on good patient adherence to the programme and continuing information about the benefit of decreasing BP. This could be possible since hypertension is now well recognized as one of the most important risk factors for the development of cardiovascular cerebrovascular and renovascular disease (2-5-6).

In conclusion the present study has shown that a majority of middle aged men with hypertension can be treated in an acceptable way without too many side effects and with a low drop-out rate.

The methods used to achieve these results are fairly simple requiring limited medical and economic resources.

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Emergency Treatment of Severe Hypertension Evaluated in a Randomized Study

*Effect of Rest and Furosemide and a Randomized Evaluation
of Chlorpromazine, Dihydralazine and Diazoxide*

Danish Multicenter Study

Organizing Committee

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ABSTRACT Emergency treatment of acute severe hypertension was monitored prospectively during a period of nine months in a Danish multicenter trial. A total of 101 patients with supine diastolic blood pressure (DBP) phase V ≥ 135 mmHg in three measurements at 5 min intervals entered the study. The emergency treatment was divided into three periods: Period I lasting for the first hour, the patient was left in the supine position and no drug treatment given. If DBP did not fall below 135 mmHg the patient entered period II during which furosemide, 40 mg i.v. was given and observation continued for the next hour. Patients with pronounced hypertensive encephalopathy ($n=15$) and left ventricular heart failure ($n=2$) started directly at period II. Provided DBP after was still 135 mmHg or higher, the patients entered period III (2nd-5th hour) to be randomized in period III (2nd-5th hour) to be treated with either chlorpromazine or dihydralazine in small refractory i.v. doses or diazoxide 5 mg/kg b.wt i.v., as a bolus injection. In period I DBP fell below our emergency limit of 135 mmHg in 5 (27%) of 84 patients whose mean arterial BP fell from 171 to 140 mmHg during the study. In period II DBP fell below 135 mmHg in 23 (30%) of 78 patients and their mean arterial BP decreased from 174 to 135 mmHg during the study. In period III 16 patients were treated with chlorpromazine, 16 with dihydralazine and 15 with diazoxide. In patients treated with chlorpromazine or dihydralazine BP decreased gradually during the first hour, on average from 211/146 to 155/103 and from 219/147 to 162/89 mmHg respectively. After diazoxide BP decreased abruptly in the first two minutes on average from 211/144 to 166/100 mmHg. The complication rate was generally low and only 4 patients were resistant to any of the treatments

given. The study was not able to demonstrate that diazoxide is preferable to the other two drugs in emergency treatment.

Key words: hypertension, hypertensive encephalopathy, treatment of hypertension, chlorpromazine, dihydralazine, diazoxide.

Acta Med Scand 208 473 1980

In recent decades several drugs have been recommended in emergency treatment of severe hypertension (1, 5, 6, 10, 15, 16). As the annual number of patients requiring emergency treatment is low for a single medical department, personal experience of such patients is often limited. These factors seem to explain the considerable variations in the recommended treatment from centre to centre.

Several reports have been published, especially from the US (1, 4, 6, 7, 8, 9, 11, 13, 15, 17, 18, 20).

Collaborating doctors: O. Amtrup, M. Brunt, J. Bukh, C. K. Christensen, P. Christiansen, T. Cortsen, J. Fischer, Hansen, M. Friedberg, R. Friedberg, K. Garde, H. Gedaj, K. Gungor, Mortensen, L. Hagerup, J. Hansen, H. Hilden, G. Hvid, H. Erenlund, Jensen, P. Jest, P. W. Jørgensen, J. Kampmann, H. Østergaard, Kristensen, O. Krogsgaard, J. Ladefoged, A. Leith, J. Lindskov, C. Vind, Ludvigsen, I. Madsen, H. Mørch, P. P. Olsen, G. Pedersen, K. E. Petersen, H. Østergaard, Petersen, V. Rasmussen, J. Røkkedal, K. Sloth, E. Taudorf, F. Trautner, H. Winzenzen.

Abbreviations: BP = blood pressure, DBP = diastolic BP, SBP = systolic BP.

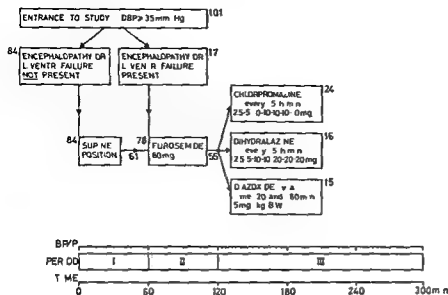


Fig. 1. Design of the study.

22) which recommend administration of the vasodilating drug diazoxide in emergency treatment of severe hypertension. In only one controlled trial (2) on hypertensive subjects with only slightly elevated blood pressure (BP) has diazoxide so far been compared to other drugs: trimethaphan and sodium nitroprusside in emergency treatment. We therefore decided to perform a multicenter trial comparing diazoxide to dihydralazine and chlorpromazine in the treatment of severe hypertension. Dihydralazine has for several years been a well known drug in emergency antihypertensive therapy, and chlorpromazine has been used for years in many medical wards in Scandinavia with satisfactory results.

This report deals with experiences of the first part of the multicenter trial comprising about 100 patients.

STUDY BASE AND METHOD

The study was performed between Jan. 10, 1977 and June 1, 1978. Thirty-nine medical departments constituting 43% of all medical beds in Denmark (15 million inhabitants) joined the study. *Positive inclusion criterion:* average supine diastolic blood pressure (DBP) ≥ 135 mmHg in three consecutive measurements at 5 min intervals. *Exclusion criteria:* a) Severe ischaemic heart disease (history of myocardial infarction, severe angina pectoris); b) Uraemia with S-creatinine above 500 $\mu\text{mol/l}$; c) Earlier or recent cerebrovascular attack (thrombosis, haemorrhage, subarachnoid haemorrhage, transient ischaemic attack).

The autoscillatory measurements were performed using

standard cuffs (12 \times 35 cm) or for patients with arm circumference over 35 cm 14 \times 45 cm cuffs (3). DBP was recorded at disappearance of Korotkoff sounds phase 5.

The study covered the first five hours and was divided into three periods (Fig. 1).

Periods I and II: During the first hour, period I, patients were in the supine position. Patients with symptoms of severe hypertensive encephalopathy or severe left ventricular heart failure, however entered the study at period II. If DBP ≥ 145 and 60 mmHg a rapid mmHg or more, the patient continued to period II and 40 mg furosemide was injected intravenously. If DBP lower than 135 mmHg, the patient remained in emergency study and BP and heart rate were checked every half hour for the following 4 hours. During period II, which lasted one hour, the same limits of BP were used to decide whether the patient should continue to period III.

Period III: The remaining patients were randomized to treatment with either chlorpromazine or dihydralazine or diazoxide. Closed envelopes, numbered consecutively, and statistical tables of randomized numbers were used.

The recommended and maximum doses of the drugs are listed in Table I. Chlorpromazine and dihydralazine were given as small bolus, inject once only until DBP had fallen to 110 mmHg or below. As pointed out in the protocol that if the BP fell rapidly, the previous injection of the drug should be reduced in order to avoid severe hypotension. Diazoxide was given in the recommended dose 5 mg/kg bolus injected intravenously running drip over 0-20 sec. If the BP was not sufficiently reduced (DBP > 110 mmHg), a new injection of diazoxide could be given one hour later. For patients allocated to treatment with diazoxide, a drip of metaraminol (Aramon[®]) should be prepared. The antihypertensive treatment after the study was not mentioned in the protocol.

Any side-effects of the treatments were described in reports as well as information about known hypertension before the study and an evaluation concerning symptoms.

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so of p a c n s e n the recommended dose

	Minutes							Maximum recommended dose during the first 1 h in period III	Doses used in the study	
	0	15	30	45	60	75	90		Mean	Range
promazine (mg)	7.5	5	10	10	10	10	10	57.5	25.3	7.5-67.5
hydralazine (mg)	7.5	5	10	10	0	0	0	87.5	16.5	7.5-17.5
doxide (mg/kg b.wt.)	5				5			2x5	360	90-600

retinal hypertension. S-creatinine was measured before and on the day after the study. ECG and the retinal vessels were evaluated on entrance to the study as well as before and during the study. Forms consistent with hypertensive encephalopathy were seen. A total of 103 patients were initially included in the study. Two patients were excluded secondarily: one who had been subcutaneously administered hydralazine and the other who had been given doxide. Data on the remaining 101 patients are given in Tables II and III. The male/female ratio was approximately 1. The age of the patients ranged from 1 to 8 years; the average age of those who stopped treatment at periods I and II was slightly higher than that of the patients who continued to period III. Only 44 of the patients had known hypertension before the study. The range of antihypertensive treatment varied among the patients from 2 weeks up to 32 years. A total of 7 patients had secondary hypertension (Table II). Symptoms consistent with hypertensive encephalopathy at entrance to the study (i.e. confusion, muzziness, somnolence, coma, nausea, vomiting, headache, convulsions, visual disturbances, paraesthesiae) were found in 44 patients (Table III). In 15 cases these symptoms were so pronounced that the patient entered the study at period II. Only two patients started at period

III on account of left ventricular heart failure. ECG abnormalities (left axis deviation, left ventricular hypertrophy and/or left ventricular strain) were demonstrated in 81 patients. In only four patients were the retinal vessels described as normal. Forty patients had severe retinal changes with fundus hypertensive III and IV according to the Keith-Wagener grading. The average S-creatinine varied in these five groups of patients from 110 to 160 $\mu\text{mol/l}$ (Table III).

Ethical considerations During the planning of the study the ethical problems involved were carefully discussed. Because three well established treatments were given, we found that the study fulfilled the criteria of Helsinki Declaration II.

Statistical tests Student's *t* test for unpaired observations, Wilcoxon matched pairs signed rank test and χ^2 test with Yates correction were used.

RESULTS

Clinical signs and blood pressure

Patients continuing to period III In 46 patients DBP decreased below the emergency limit (135

Table II Age, sex, distribution and form of about 100 patients on

	Known hypertension on admission										
	No. of patients			Age (y)		Duration of antihypertensive treatment (y)				Symptomatic hypertension	
						Mean		Range			
	Total	♀	♂	Mean	Range	No.	% of total	Mean	Range	No.	% of total
Period I	3	11	17	5.1	4-74	1	5	6.7	<1.3	1	4
Period II	71	11	17	57.7	37-87	13	57	5.9	<1.0	6	11
Period III											
Chlorpromazine	4	12	12	50.0	36-70	7	9	3.6	<1.11	5	1
Hydralazine	16	11	10	47.0	5-65	6	38	1.5	<1.4	3	19
Doxazosin	15	7	8	46.8	1-71	6	40	9.1	<1.3	7	47
Total	101	47	54	51.8	1-87	44	43.6	5.4	<1.3	7	71.8

Table III Hypertensive organ damage at entrance to the study

	Total no of pats	Hypertensive encephalopathy						Retinal fundus Keith Wagener			
				Pats entering at period II		ECG abnormalities		0→II		III+IV	
		No	%	No	%	No	%	No	%	No	%
Period I	23	9	39			18	78	19	100	4	
Period II	23	8	35	4	17	16	70	17	74	6	
Period III											
Chlorpromazine	24	14	58	5	21	22	92	11	46	13	
Dihydralazine	16	8	50	4	25	13	81	4	25	1*	
Diazoxide	15	5	33	2	13	12	80	10	67	5	
Total	101	44	43.6	15	14.9	81	80.2	61	60.4	40	

mmHg) during period I (23 patients out of 84=27%) or period II (23 out of 78 = 30%) (Fig. 2). A further decrease in BP was observed during the next hours. At the end of the study average BP was almost the same in the two groups 190/115 and 177/113 mmHg compared to 225/144 and 236/143 mmHg respectively at entrance. Thus the decrease in mean BP during the 5 hours of observation was 37 and 40 mmHg respectively in the two groups.

Patients continuing to period III. Fifty five patients were allocated to treatment with either chlorpromazine (24), dihydralazine (16) or diazoxide (15) (Fig. 3). BP decreased gradually with chlorpromazine and dihydralazine during the first hour of period III with no significant difference between the two groups (Table IV). The individual doses of the two drugs varied considerably (Table I) the average dose of chlorpromazine being 25.3 mg

(range 7.5-57.5) and of dihydralazine 16.5 mg (range 2.5-47.5). The maximum recommended dose of chlorpromazine was given to only two patients while none of the patients received the maximum recommended dose of dihydralazine. In 27 of the patients in these two groups BP was already lowered sufficiently after the first three injections.

In the group treated with diazoxide (Fig. 1, T IV) an immediate drop in BP was observed one min after the injection (from 218/144 to 166 mmHg) during the next hour it increased slightly. Only two patients received an injection of diazoxide one hour after the initial injection. The average 3, 4 and 5 hours after entrance to the study showed no statistically significant differences between the three groups of patients treated during period III (Table IV).

The individual responses to emergency treatment varied considerably. After the initial 90 min period III 37 of the 55 patients had a DBP of 135 mmHg or below (Fig. 4). Four patients still had DBPs above the emergency limit (≥ 135 mmHg) three in the chlorpromazine and one in the dihydralazine treated group while none of the diazoxide treated patients had BPs above this limit.

Severe pressure drop. (systolic BP (SBP) < 100 mmHg) was observed in 12 patients (4 in the chlorpromazine, 2 in the dihydralazine, 6 in the diazoxide group) (Table V). The differences between the three groups are not statistically significant. In 2 of the patients treated with chlorpromazine the fall in BP seemed to be explained by vasovagal attacks as the heart rate decreased considerably.

Secondary pressure rise later during the hour

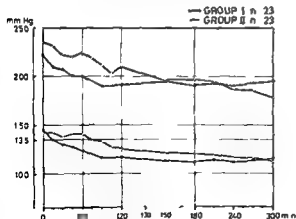


Fig. 2 Changes in SBP and DBP in patients responding to treatment with supine position alone (group I) or supine position + furosemide (group II)

C (μmol)	4 h after the study	
	Mean	Range
18-305	140	88-305
70-34	177*	63-18
0-34	152*	70-373
0-90	141	65-90
84-16	184	85-394
0-362	146.4	63-394

with DBP increasing to 135 mmHg or more the final emergency study was demonstrated of the 46 patients who left the study during I or II and 76 of the 55 who continued to treatment in period III ($\chi^2 3.8$ $p < 0.01$)

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significant increase in heart rate was found in the patients treated with dihydralazine. Only a small non significant increase was found among those given diazoxide or chlorpromazine. Severe tachycardia with heart rate > 140 was observed in two patients treated with dihydralazine (Table V). The two patients who developed bradycardia due to vasovagal attacks during chlorpromazine therapy were treated successfully with atropine.

GROUP III
 — CHLORPROMAZINE n 24
 — DIHYDRALAZINE n 6
 — DIAZOXIDE n 5

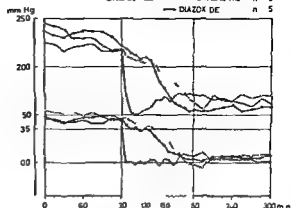


Fig. 3 Changes in SBP and DBP in patients allocated to treatment during period III.

Complications and side effects

The incidence of early complications and side effects is given in Table V. No complications were seen in the 46 patients who left the emergency study during period I or II. The 17 patients with severe pressure drop during period III have already been mentioned. Eight patients complained of nausea, vomiting and abdominal pain during period III. No cardiac complications were observed apart from precordial pain in two patients treated with dihydralazine. A 53-year-old man developed a hemiparesis during treatment with dihydralazine in the first hour of period III. His BP decreased steadily during this hour from 250/150 to 120/80 mmHg. While still in hospital he obtained remission of the

Table IV Blood pressure (mmHg) measured during period III (mean \pm SD and range (in parentheses))

Random group	10 min	160 min	40 min	300 min
Chlorpromazine (n = 24)				
SBP	146.9 (130-270)	155.40 (100-211)	160.3 (110-200)	158.9 (110-250)
DBP	103 (70-145)	103 (70-145)	107.18 (80-145)	103.17 (80-140)
Dihydralazine (n = 16)				
SBP	147.11 (130-250)	163.81 (105-200)	165.31 (90-210)	170.33 (110-250)
DBP	108.18 (70-170)	98.18 (65-145)	105.19 (65-140)	108.18 (80-130)
Diazoxide (n = 15)				
SBP	144.10 (130-250)	171.37 (110-211)	165.31 (110-210)	153.43 (100-200)
DBP	107.14 (85-130)	107.14 (85-130)	104.15 (85-140)	101.18 (75-135)

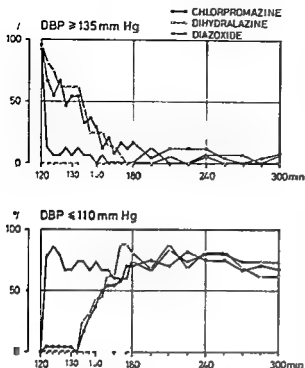


Fig 4 Percentage of patients with DBP ≥ 135 or ≤ 110 mmHg during period III

hemiparesis but at discharge a slight paresis was still demonstrable. Cerebral arteriography was not performed. In a 50-year-old woman arm paresis and paresis of the facial nerve on admission deteriorated during treatment with chlorpromazine followed by remission. Her BP decreased from 245/155 to 95/60 mmHg during the first 45 min in period III. The total dose of chlorpromazine administered to this patient was only 7.5 mg.

Changes in renal function

A significant increase in S-creatinine was observed in all five groups during the first 24 hours (Table III).

Late complications

Six patients died later during the hospital stay, four primarily due to intracerebral haemorrhage, cerebral oedema, and one patient each of myocardial infarction and severe uraemia. One patient developed secondarily severe encephalopathy, subarachnoidal haemorrhage followed by complete remission.

DISCUSSION

In the present study a DBP level of ≥ 135 mmHg was used as indication for emergency treatment. Such a rigid limit is of course debatable, especially as many of the patients were not affected by high BPs. On the other hand, a multicenter study requires a protocol that is not too different.

This study was initiated in order to answer the following three questions: 1) How often can a very elevated BP be treated by just placing the patient in the supine position, possibly followed by furosemide? 2) If further treatment is needed, how efficient is chlorpromazine, dihydralazine, or diazoxide? 3) Is it possible on the basis of these results to decide which of these drugs should be preferred in emergency therapy?

The study showed that about one fourth of the patients reached a clinically acceptable BP merely by being placed in the supine position for one hour. A further one fourth reached an acceptable BP after 1-2 v furosemide. In none of these patients were complications recorded during the emergency treatment or later during the hospital stay.

Compared with group III, the patients in groups I and II included a significantly lower proportion with pronounced retinopathy (Table III) and a less severe form of hypertension. Earlier severe hypertension was also more frequent in groups I and II than in group III.

Table V Complications and side effects during the study

	Total no of pts	No. of complications and side-effects						I.
		SBP ≤ 100 mmHg	Nausea vomiting sweating	Pre cordial pain	Tachycardia ≥ 100	Bradycardia < 50	Hemiparesis	
Periods I and II	46							
Period III								
Chlorpromazine	24	4	2			2		7
Dihydralazine	16	2	3	2	2		1	9
Diazoxide	15	1	3					1

Three emergency treatments used in the present study were effective in lowering the markedly raised BP as only few of the 55 patients could be categorized as non responders. The hypotensive effect of dihydralazine and diazoxide is well known by many clinicians outside the Scandinavian countries. It is probably not aware that the α blocking effect of chlorpromazine (21) is useful in hypertension in emergency. Comparison of the three drugs showed a slow and quite similar decrease in BP in the groups treated with dihydralazine and chlorpromazine respectively. In contrast diazoxide (5 mg/kg b.wt) was followed by an immediate fall in BP on average 2 min after the i.v. injection corresponding to the findings of several other authors (7, 12, 13, 17, 18, 20, 22).

The immediate effect of diazoxide followed by a persistently lowered BP for the next hours gives it advantages compared to the repeated bolus injections of chlorpromazine and dihydralazine. On the other hand repeated injections of the other two drugs yield valuable information about the sensitivity of the patient (Table I). Some clinicians have noted that repeated injections are time-consuming. However the present study showed that using the recommended dose of chlorpromazine or dihydralazine about 2/3 of the patients (27 of 40) reached normotension in DBP after only three injections given at intervals of 15 min.

The number of patients in the present study is too small to show whether one of the three drugs is preferable to the others in terms of a lower complication rate. As a whole the complication rate was very low. The vasovagal attacks were found only among patients treated with chlorpromazine but according to the literature this has also been found among patients treated with diazoxide as well as dihydralazine (14, 19). The patient who developed a paraparesis during period III may have had an intracerebral pressure rise after an intracerebral attack. However the other possibility exists that administration of dihydralazine increased the intracerebral pressure with a secondary decrease in intracerebral flow as recently shown in patients with traumatic head injuries (24).

Severe hypertensive encephalopathy is probably excited by a break through phenomenon i.e. the cerebral blood flow is constant over a wide range of pressures but at a very high sustained pressure cerebral blood flow will increase followed by cerebral oedema. It is not yet known whether it is preferable

to reduce the BP slowly or rapidly. The autoregulation of the cerebral vessels is probably able to counteract even rapid changes in BP such as were seen in patients treated with diazoxide (12). The lower limit of the autoregulation curve seems to be shifted to higher levels among hypertensive than normotensive subjects (26) i.e. symptoms consistent with cerebral anoxia might be demonstrated at higher BP levels among the former. Severe hypotension should therefore be avoided during emergency treatment. No symptoms of cerebral anoxia were noted among our 12 patients who developed hypotension during period III (SBP < 100 mmHg). On the other hand a sudden severe reduction of SBP might be potentially dangerous not only to the cerebral circulation but also to the myocardial and renal circulation (14). So far no comparisons of cerebral haemodynamics have been performed using different emergency treatments in severe hypertension (26).

Diazoxide is often recommended in the Anglo-Saxon literature as the drug of first choice in emergency therapy due to its immediate and long acting effect, very low incidence of complications and side effects and very low failure rate (1, 3, 8, 9, 21). This recommendation seems to be based on clinical experience not on controlled trials. In the present study chlorpromazine and dihydralazine were both quite as effective as diazoxide in lowering the BP. Furthermore it is fair to conclude from this study that the recommended dose of diazoxide (5 mg/kg b.wt) is too high for a rather large group of the patients. It has been shown in hypertensive children (3) that diazoxide might be given as smaller i.v. bolus injections (1–2 mg/kg b.wt) repeated every 10–15 min. Recently Ram and Kaplan (25) found individual titration of diazoxide 140 mg every 5 min effective in controlling severe hypertension. Our preliminary results agree with the results of their study.

ADDENDUM

When we had finished this part of the study two British groups reported on 12 patients with severe neurological complications or even fatal cases after hypertensive emergency therapy (Cove et al. *Br Med J* 245: 1979; Ledingham & Rajagopalan *Q J Med* 48: 25: 1979). The publications have been discussed extensively in leading articles in *Br Med J* and the *Lancet*. The case reports showed that rapid

reduction of high BP might be dangerous especially if hypotension or even normotension is achieved. In our opinion the majority of the patients had been overtreated whether using diazoxide in a large i.v. bolus injection or diazoxide combined with phenothiazine derivatives such as chlorpromazine. However, one of the patients developed irreversible blindness even after only peroral medication with a slow reduction of BP over three days.

It seems important to stress that emergency therapy might be dangerous and that the BP should be lowered steadily and not to normotensive values within the first days after initiation of the therapy.

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Borderline Hypertension

Hypertension Seminars at Ostra Hospital Goteborg Sweden

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ABSTRACT Borderline hypertension was the topic of the "Hypertension seminars" arranged by the Hypertension Section at the Ostra Hospital, Goteborg, Sweden. On that occasion Professor Stevo Julius Ann Arbor, Michigan, USA, was an invited speaker. During the seminar, various aspects of borderline hypertension were discussed, e.g. the natural history, hemodynamics and management of this condition. In the present review we are based on these discussions.

KEY WORDS: borderline hypertension; hemodynamics; epidemiology.

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It has been shown beyond doubt and there is general agreement today that arterial pressure in any population is distributed as a continuous variable, i.e. being Gaussian in shape when converted to a logarithmic scale. In other words, arterial pressure is a quantity, not a quality. With this in mind, it is not logical to treat arterial pressure as a dichotomy, i.e. normotension and hypertension, a fact repeatedly pointed out by Sir James Pickering (75-76).

The fact that most physicians and scientists still use the terms hypertension and normotension and the term borderline hypertension, the topic of this seminar, does not mean that they do not recognize that blood pressure (BP) is a quantitative variable. The use of these terms reflects the realization that individuals with a certain level of BP have a different common physiologic and epidemiologic characteristics which often mandate specific investigations and practical approaches. As will become clear during this seminar, the term borderline hypertension signifies not just a certain range of BP between normotension and hypertension, but

rather a clinical condition with recognizable and reproducible characteristics.

DEFINITIONS

The WHO has defined hypertension as systolic BP (SBP) ≥ 160 and/or diastolic BP (DBP) ≥ 95 mmHg and normotension as $\leq 140/90$ mmHg, leaving the range between these two delineations to be called borderline hypertension (101). The Ann Arbor group has previously defined patients with borderline hypertension as those having two out of five indirect casual BPs within the last year at least one with a diastolic value ≥ 90 mmHg and at least one ≤ 90 mmHg (45). Others have defined borderline hypertension as BP intermittently above 150 mmHg systolic or 90 mmHg diastolic (94). Since arterial pressure tends to increase with age in the Western world, all the above definitions have inherent problems, simply because they do not consider the age of the subject. However, the Ann Arbor group has recently taken age into consideration and defines borderline hypertension in the following manner: age 17-40 $>140/90$ $<160/100$, age 41-60 $>150/90$ $<160/100$ and age >60 $>160/90$ $<175/100$ (40). Obviously the intent of all these classifications is to delineate the mildest possible form of hypertension. Consequently, the term borderline hypertension excludes the existence of BP-related target organ damage such as hypertensive retinopathy and left ventricular hypertrophy (with conventional methods) or impaired renal function.

A number of synonyms to borderline hypertension have been proposed. The term labile hypertension is not logical as it implies increased BP variability. Some of the early studies indicating a higher degree of BP variability in subjects with systolic blood pressure above 120 mmHg as compared to those with BPs below this level are open to serious criticism, e.g. that in the lower BP group the upward variability of the BP was limited by definition (it could reach a maximum of 120 mmHg) whereas no such restrictions existed in the high BP group (78).

Furthermore, several studies have demonstrated that there is no correlation between the level of BP and its variability when repeated measurements are made during 1-3 weeks (10, 26). In addition, BP fluctuates markedly

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even in normotensive individuals (4). It is therefore not surprising to find that an excessive variability of BP has never been established as a characteristic feature of borderline hypertension. Even if the BP variability were increased the importance of this finding would be questionable. The extreme ranges of BP do not seem to predict the risk of cardiovascular disease. Thus Sokolow et al (85) found that the five highest and five lowest BPs during a 24-hour recording did not relate to cardiovascular morbidity whereas the average BP during that period carried an important predictive power. The term 'labile' should be reserved for individuals with extremely variable BPs irrespective of whether these occur in the normotensive borderline or hypertensive range.

The term prehypertension indicates a condition which almost invariably leads to established hypertension. Since this is not the case which will be shown later the term is not logical. Latent hypertension points to a condition which may develop into hypertension or which may remain latent and this term is accordingly a useful synonym. Marginal hypertension is also acceptable whereas early essential hypertension should be avoided for the same reasons as prehypertension. However borderline hypertension is such an accepted and commonly used expression that any attempt to use synonyms for this condition is likely to cause confusion. For this reason we would recommend that the use of synonyms be restricted.

EPIDEMIOLOGY

The prevalence of borderline hypertension has been studied in several population surveys. In the Bergen study performed in the 1950s 8% of the males in the age group 20-30 years had borderline hypertension and 13% in the 30-40 years group. The prevalence of borderline hypertension was somewhat lower in women below 50 years of age but with increasing age this difference disappeared (6). Also in the US the prevalence of borderline hypertension increases rapidly with increasing age (7). The prevalence of borderline hypertension in university students in Michigan has been reported to be approximately 20% (40).

Accurate data about the prevalence of borderline hypertension in Sweden are lacking. However if the prevalence data from the Alameda County BP study in California were valid also in Sweden the total number of persons with borderline hypertension would exceed one million (of a total population of 8 millions) (Table I).

For a complete review of available data on the prevalence of borderline hypertension see Julius and Schork (47). In this context it should be stressed again that the definition of borderline hypertension and indeed also of hypertension becomes crucial with advancing age. For example a

Table I Calculated number of persons with borderline hypertension in Sweden

Based on population data on Dec. 31 1976 the population of Sweden being 8236179 (40) and Alameda County BP study (7)

Age group	Males	Females
18-24	19 900	7 600
25-44	114 200	54 400
45-64	163 100	196 700
>65	106 800	357 400
All	403 800	616 000
All males and females	1 019 800	

population study of 70-year old women in Göttingen indicated that no less than 48% had hypertension (92) demonstrating the dilemma of arbitrary definitions of hypertension. The incidence of borderline hypertension has repeatedly been found to be quite low in the order of 1%/year of observation (47-52).

NATURAL HISTORY AND RISK FACTORS

The later occurrence of established hypertension has repeatedly been found to be 2-5 times as common in individuals with borderline hypertension than in those with initial normal BP. It should be stressed that only a minority of individuals with borderline hypertension later develop established hypertension. An approximate figure for the accumulated overall risk is commonly considered to be around 20% (44-70, 73).

The morbidity and mortality from cardiovascular disease in the population with borderline hypertension is approximately twice as high as in a normotensive population (47). The increased morbidity in borderline hypertension shows a similar pattern to the morbidity observed in established hypertension. Thus an excess in myocardial infarcts, strokes, congestive heart failure and ECG changes has been reported (32, 51, 57, 62, 68, 90). It is consequently likely but not proven that the earlier morbidity occurs in the group (20%) of persons who will later develop established hypertension (hypertension specific cardiovascular morbidity).

A good example of morbidity in borderline hypertension can be found in the Framingham study which showed a 50% increase in age adjusted coronary heart disease (50, 51). Other studies have shown similar or higher (up to 200%) in recent

early morbidity in borderline hypertension (39). The Framingham study also found an almost doubled incidence of atherothrombotic brain lesions.

The mortality in borderline hypertension is also increased. One could question the specificity of this finding since in the report by Levy et al. (57) subjects with borderline hypertension had a greater tendency to commit suicide. However, the Framingham study shows that the excess mortality in borderline hypertension is specific.

A 40% increased overall mortality for men and a 25% increase for women with borderline hypertension as compared to normotensive control groups is reported. The increase is largely due to cardiovascular mortality, which was twice the normal for men and four times that for women in age group 67-74 (50). In younger age groups the increase in cardiovascular mortality was substantial but not as dramatic as in older ages (50).

As the risks for complications increase with rising blood pressure, it is not surprising that a great deal of work has been interested in attempts to identify factors that predict future hypertension. As it happens, the best predictor for future hypertension is the initial blood pressure level; the strongest predictor for blood pressure in the future (11, 32, 48, 64, 69, 87). Other independent predictive factors are tachycardia, which is not a benign sign (70, 87). Levy et al. found that when both transient hypertension and transient tachycardia were present, the incidence of established hypertension was more than twice the incidence when only one of these factors was present (58).

Overweight is also associated with borderline and established hypertension. High relative weight is a predictor of future hypertension, as is weight gain over a period of years (32, 37, 38, 70, 87, 89).

A positive family history of hypertension is more common in patients with borderline blood pressure elevation than in established hypertension, which occurs more often in patients with a positive family history (29). The inheritance of arterial pressure is considered to be multigenetic but the exact mechanisms are not known (80, 97).

Interest has recently been focused on possible interactions between environmental and genetic factors in the development of hypertension (12, 93). The question has been asked whether there are genetic indicators—e.g. certain blood types—at least in some subpopulations in es-

sential hypertension. If so, it might be possible to predict the prognosis or the risk for e.g. vascular damage more reliably than is presently possible (27, 53). However, to our knowledge, no such studies have been performed systematically in individuals with borderline hypertension.

In the overall US population, the prevalence of borderline hypertension is very similar for black and white persons of both sexes, but established hypertension is twice as frequent in blacks, indicating that race may be considered as a risk factor and that blacks carry a greater risk for progression from borderline to sustained hypertension (40).

Using multiple logistic function to discern the quintile of the population with the highest risk of developing hypertension within 5 years, Stamler et al. (87) could identify at most 55% of all expected hypertensives in that quintile.

A number of tests have been designed and employed in attempts to identify persons with an augmented risk of developing future hypertension, e.g. cold pressor test, salt loading reaction, to mental arithmetic, digital vascular reactivity, to noradrenalin, apparent noradrenalin secretion test and initiated noradrenalin test, as well as different combinations of these. By and large, such tests have proved to be of no predictive value, or alternatively, they cannot be employed in practice or the needed long term follow up is still lacking (40, 91). This holds true also for a new and most interesting approach, the abnormal Na^+ and K^+ net fluxes in erythrocytes of essential hypertensive patients (also in relatives to hypertensive patients and in some women with hypertension during pregnancy) (25). Whether these abnormalities can really be used as markers for hypertension remains to be confirmed.

HUMORAL FACTORS

Catecholamine concentrations in urine or plasma are frequently used as indicators of sympathetic activity. It is, however, doubtful that patients with established hypertension have increased sympathetic activity, as reflected by increased release of catecholamines (8, 15, 18, 19, 23, 34, 35, 59, 68, 74, 81). Age is of importance in this setting, since plasma concentration of noradrenalin is positively correlated with age (23, 34, 74, 81). Activation of the cardiac sympathetic neurones increases urinary catecholamine excretion (9), whereas moderate activation of the peripheral sympathetic ner-

Table II Hemodynamics in borderline hypertension

	Controls (n=29)	Whole patient group (n=44)	Hyperkinetic subgroup (n=20)	Normotensive subgroup (n=24)
Blood pressure (mmHg)				
SBP	120	137**	138 *	136*
DBP	71	75**	76 *	74
Mean	88	95**	97 *	94
Heart rate (beats/min)	65	69	76* *	Δ 63
Stroke volume (ml)	98	111**	119 *	Δ 104
Cardiac output (l/min)	6.1	7.3**	8.6**	ΔΔΔ 6.9
Cardiac index (l/min m ²)	3.33	3.81*	4.55**	ΔΔΔ 3.19
Total peripheral resistance (mmHg/l/min)	14.8	13.4*	11.2**	ΔΔΔ 15.1
Minimal vascular resistance in the hands (mmHg/(ml/min 100 ml))	1.69	1.87*	1.71	Δ 1.99
Body weight (kg)	63.9	73.8**	71.4*	73.6

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ in comparison with controls Δ $p < 0.05$ ΔΔ $p < 0.01$ ΔΔΔ $p < 0.001$ in comparison with hyperkinetic subgroup (Sivertsson et al. Unpublished data)

system does not increase plasma concentrations of adrenalin or noradrenalin (1, 61). Some patients with established hypertension appear to have a true neurogenic hypertension and their BP can be normalized following total autonomic blockade (16). Hypertensive patients showing higher noradrenalin levels are usually young (23, 34, 81) and have also been reported to have increased plasma renin activity (16).

In comparison with normotensive individuals borderline hypertensives usually have normal resting plasma and urinary catecholamine concentrations (8, 67). Physical exercise induces elevation of both noradrenalin and adrenalin plasma concentration but patients with borderline hypertension do not behave differently from normal controls in this respect. Patients with borderline hypertension have increased excretion of urinary catecholamines during mental stress (19, 67) and also an increased catecholamine response to upright posture (19, 44). Poststress plasma catecholamines have recently been shown to be increased also in individuals with a genetic risk of developing hypertension as well as in borderline hypertensives (20).

As a group borderline hypertensives have higher plasma renin activity than normotensive individuals and patients with established hypertension (24). Patients with borderline hypertension have been subdivided by their renin status (17) and approximately 35% are in the high renin group, 45% in the normal renin group and 20% in the low renin group (17). Patients with hyperkinetic circulation usually have high plasma renin activity (65).

Total autonomic blockade with propranolol, ropine and phentolamine normalizes the BP in patients with high renin borderline hypertension and in high renin established hypertension (16). However, in patients with normal or low renin hypertension the BP remains elevated also following total autonomic blockade (17). This would indicate that the high renin hypertension is neurogenic type of BP elevation. Obviously plasma renin activity per se does not induce the elevation in these patients, since blockade with propranolol, which causes a marked decrease in plasma renin levels, does not reduce elevated BP (43).

PERSONALITY TRAITS

A few reports indicate that patients with borderline hypertension differ from normal controls in a number of psychological ways (28, 30). They are more submissive and have more suppressed hostility (28). They also more often exhibit imbalances in sexual roles and have difficulties in interactions with other people (49). These data indicate that borderline hypertension could be regarded as a psychosomatic disease.

HEMODYNAMICS

Cardiac output

It is well documented that cardiac output is increased in patients with borderline hypertension as a group in comparison with normotensive

(47) However this does not mean that all individuals with borderline hypertension have an increased cardiac output. But in 30–50% the cardiac output exceeds the value found in control subjects.

SD or more. This difference between border hypertensives and normotensives seems to decrease with increasing age (41–60). Some studies state that the high output is caused by an elevated heart rate (24–41, 60–63, 79), others that it is due to an increased stroke volume (3–21) while still others have demonstrated that both factors are involved (13, 78–100) (Table II).

The elevation of cardiac output is maintained by increased sympathetic and a reduced vagal activity to the heart which points to an altered central nervous activity in these individuals. Thus cardiac output normalized after autonomic blockade with propranolol and atropine (45). Since the sensitivity of the β adrenoceptors in the myocardium is depressed rather than increased in borderline hypertension the high output state was not caused by adrenergic effects (43).

Blood volume

Most studies show that blood volume or plasma volume is normal or slightly reduced in borderline hypertension (2–21, 24–63) while a few studies show significantly reduced values (22–46, 78). Cardiac pulmonary blood volume is probably normal in patients with borderline hypertension (78). In relation to total blood volume, cardiopulmonary blood volume has been found to be normal (79) or increased (78). In patients with hyperkinetic border hypertension the cardiopulmonary blood volume appears to be increased both in absolute and in relative terms (14). This centralization of blood from periphery is probably a consequence of an increased vasomotor nerve tone on the capacitance vessels (78). Such a redistribution of the blood pool might influence stroke volume and cardiac output by increasing the venous return. Although there is a positive correlation between cardiac output and cardiopulmonary blood volume (14) this does not have a causal connection since vasomotor nerve activity may influence both these variables. On the contrary, some data indicate that the two factors are not causally related. Thus, autonomic nerve blockade normalizes cardiac output but not the central blood volume (14). A changed relation between central blood volume and stroke volume or cardiac output (increased stroke volume in relation to central

blood volume) supports the idea that vasomotor nerve activity is the main determinant of the increased cardiac output in patients with borderline hypertension (14).

Peripheral vessels and vascular resistance

At rest total peripheral resistance in borderline hypertension is within normal limits or in fact slightly reduced (Table II). It has been pointed out though that in relation to cardiac output, vascular resistance is increased (46). The vascular resistance can be brought into the normal range by means of phenolamine in about 30% of patients with borderline hypertension (43). During intense physical exercise total vascular resistance in borderline hypertension remains elevated (60–79). Moreover during infusion of dextran the vascular resistance in borderline hypertension fails to adjust to the increased cardiac output (45).

Adaptive structural changes in the resistance vessels seem to occur already in mild forms of hypertension (83–84) (Table II). This is in agreement with the echocardiographic observation of left ventricular hypertrophy in borderline hypertensive patients (56). A recent study has shown decreased venous distensibility in borderline hypertension (94). The claim was made that this decreased distensibility was to a certain extent due to structural changes in the veins (94). However the scientific support for this claim is not convincing and the findings may be explained by increased smooth muscle tone.

MANAGEMENT

It has been pointed out above that borderline hypertension constitutes an important risk factor for future cardiovascular complications. A logical consequence of this would be to lower the BP. However available data so far, notably the Veterans Administration studies, have shown positive therapeutic effects as regards morbidity and mortality only in patients whose DBPs were 105 mmHg or higher (98–99). However the HDFFP (Hypertension Detection and Follow up Program) study (35–36) demonstrates for the first time a clear reduction in mortality also in patients whose DBPs were 90–105 mmHg. Obviously these important new results will have a profound effect on management policies for patients with mild hypertension.

Before any kind of intervention is considered in

patients with borderline hypertension it is obvious that the BP level should be determined with the greatest possible accuracy. Therefore, repeated measurements of BP with a reliable and standardised technique become mandatory.

Borderline hypertensive patients are frequently overweight and since weight reduction lowers BP in obese hypertensive patients (33) it becomes obvious that reduction of body weight should be attempted in borderline patients too. The BP lowering effects of weight reduction have been shown to be independent of salt restriction (77). Still, salt reduction of a moderate degree will also lower BP. A reduction of daily salt intake from about 10 to 5 g may reduce the BP approximately 10/5 mmHg (66, 71). Dietary advice may also be beneficial in other ways, e.g. by correcting hyperlipidemia and glucose metabolism disturbances.

In a review of non pharmacological treatment of hypertension Blackburn (5) pointed out that

Exercise can be recommended to mildly hypertensive patients as to the entire public for its long term benefit in weight control in a sedentary society. However, it is not established that cardiovascular mechanisms involving autonomic nervous activity which are affected by high level conditioning exercise have in fact any independent or long term antihypertensive effect.

Behavioural methods to reduce high BP include biofeedback, relaxation, psychotherapy, environmental modification and placebo. In an extensive review of this field Shapiro et al. (82) concluded that widespread and uncritical application of behavioural methods in the treatment of high blood pressure is premature.

In the management of patients with hypertension it is obvious that advice and information play important roles. However, such information should be given with care. Canadian steel workers tended to have longer periods of non attendance when aware of a hypertensive condition than controls who were not aware of having similar BP levels (31).

In conclusion, non pharmacological treatment and regular check ups of BP constitute the basis for managing borderline hypertensive patients. Drug therapy is not recommended in cases of truly borderline hypertension, i.e. with DBPs sometimes below, sometimes slightly above 90 mmHg. However, the recent findings of the HDFP group clearly support the opinion that pharmacological treatment should be instituted in hypertensive patients, even

in the mid range, i.e. with DBPs between 90 and 105 mmHg (35, 36). Furthermore, treatment, particularly with a β adrenoceptor blocking agent, should be considered in borderline hypertensive patients with subjective symptoms related to their condition, e.g. disturbing palpitations or tachycardia.

It also stands to reason that treatment should be attempted in those patients with borderline hypertension who are at high risk to develop full hypertension due to their family history, race, uncorrectable other risk factors. Only larger doses of drugs and only monotherapy are recommended in such cases. It would be unwise to apply the same aggressive stepped-care approach to treatment that is usually used in established hypertension.

CONCLUSIONS

Borderline hypertension refers to that group between clearly normotensive and hypertensive values. It is interesting to note that a number of pathophysiological characteristics make it possible to separate borderline hypertension on the one hand from normotension and on the other from established hypertension. All the available data indicate that borderline hypertension constitutes the first phase in the development of established hypertension, although it should be pointed out that individuals with BPs within the borderline range by means are bound to develop established hypertension. It is also worth noting that BPs within the borderline hypertensive range are associated with increased risks for cardiovascular morbidity and mortality. The newly published and clearly positive results of the HDFP study regarding antihypertensive therapy in mild hypertension will probably contribute to increased attention of this clinical entity.

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Amiloride-Induced Hyponatremia

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TRACT We present three aged female patients fulfil the criteria of a syndrome that we call "amiloride hyponatremia". They became hyponatremic during amiloride + hydrochlorothiazide therapy. They needed diuretic therapy and tolerated hydrochlorothiazide with potassium supplementation as potassium chloride. Patients with hyponatremia during diuretic therapy for cardiovascular diseases can be allocated to three groups. 1) Patients with fluid and water retention due to severe impairment of cardiac function and decreased water clearance need diuretic therapy and water restriction. 2) Patients with 'normal diuretic hyponatremia', hyponatremia and extracellular volume contraction do not be associated with this phenomenon, and the therapy involves discontinuing the unnecessary diuretic treatment. 3) Patients with amiloride hyponatremia. They need diuretic treatment and hydrochlorothiazide therapy, but the hyponatremia can be corrected by changing amiloride to potassium supplementation. 'Amiloride hyponatremia' is considered to be due to a direct effect of amiloride + hydrochlorothiazide on the distal nephrons. The combination amiloride + hydrochlorothiazide must be used cautiously in elderly patients and the possibility of hyponatremia should be born in mind in cases of symptoms and CNS disturbances.

Key words: hyponatremia cardiovascular diseases diuretic therapy amiloride therapy

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Hyponatremia during conventional diuretic therapy is a well documented but rather rare entity. It occurs in patients who show no signs of clinical heart failure or edema. It is normally accompanied by hykalemia and increased ADH activity and is usually corrected within a few days after discontinuation of diuretic treatment (1-9). Remission has also been reported following massive potassium supplementation without discontinuation of diuretic therapy (1).

Three cases of severe hyponatremia appeared over a brief period in our clinical practice. They

had all been treated with amiloride + hydrochlorothiazide. The incidence was higher than could be anticipated for normal diuretic hyponatremia and the serum potassium changes were different. Therefore we considered the possible role of amiloride in the generation of hyponatremia.

PATIENTS AND METHODS

The three patients were from the district of Mikkeli Central Hospital serving a population of about 110 000. The population is divided among health centers that are responsible for primary care. Amiloride + hydrochlorothiazide combination is fairly common as primary therapy in hypertension and heart failure.

The patients were hospitalized until the diagnosis of primary hyponatremia was established and therapy instituted. They were later invited to re-examinations. The purpose of the examinations was explained to all patients who showed good compliance in order to discover the cause of their hyponatremia.

All serum and chemical measurements were performed in the Clinical Laboratory of the hospital. All patients underwent the following examinations: renal function tests (s creatinine, creatinine clearance and urine sediment), thyroid function tests (s T₃U and s T₄), p-cortisol in the morning and afternoon and thorax X-ray during the acute phase and in examination period. Only the abnormal findings are presented here.

Potassium supplementation in this presentation stands for KCl in slow release form 13 mmol/tablet.

CASE HISTORIES

Case 1

A 68 yr old female had been on digoxin, β blockade and one tablet Diurex® (5 mg amiloride + 50 mg hydrochlorothiazide, Orion, Finland) for congestive heart failure and hypertension. Earlier she had suffered a transient hemiparesis. She was admitted to the Department of Surgery Central Hospital due to epigastric pain and vomiting in Nov. 1978. On admission she was disorientated as to time and place, the abdomen was distended, the bowel sounds were a bit tighter than normal but there were no cardiac respiratory symptoms or parietic signs. Blood pressure (BP) was 190/110 mmHg, B 116-140 g/l, B leuc 8.0 $\times 10^9$ /l, B gluc and s transaminases were within normal

1 mts. In Astrup values pH was 7.48, BE +3.5 and $p\text{CO}_2$ 39 mmHg. s-K was 2.9 mmol/l and s-Na 111 mmol/l.

During the following five days she received 1710 mmol sodium as saline infusions. The abdominal symptoms cleared and s-K values were corrected within one day but her diuresis and hyponatremia persisted. The amiloride + hydrochlorothiazide combination was then regarded as the cause of hyponatremia and was withdrawn. In a couple of days the diuresis cleared and she was referred back to the Health Center ward. Within 12 days s-Na was 178 mmol/l.

A few weeks later she developed signs of congestive heart failure and was again given Durex[®] one tablet daily. This medication was followed by hyponatremia. When amiloride was changed to potassium supplementation the electrolytes remained within normal limits and the heart compensated at dosages of 100 mg hydrochlorothiazide + 52 mmol potassium.

Five months later the patient was admitted to the Central Hospital in order to confirm the hyponatremic effect of amiloride. She had used the above mentioned thiazide + potassium combination in addition to digoxin. She showed signs of general arteriosclerosis but was otherwise well. S-K was 3.7 mmol/l and s-Na 135 mmol/l. Thiazide + potassium was changed to Durex 2 tablets daily (i.e. 100 mg hydrochlorothiazide + 10 mg amiloride). The daily urinary sodium excretion was 110 mmol before and 158 two days after the change while the corresponding daily potassium excretion decreased from 96 to 84 mmol. S-K, s-Ca, s-Pi, s-Mg and B-Hb remained within the normal limits but s-Na decreased to 125 mmol/l within 12 days. The experiment was discontinued because the patient began to show the same kind of mind lability as during earlier hyponatremic periods. Durex was changed back to the corresponding daily dose of 100 mg hydrochlorothiazide + 52 mmol potassium. One day later the daily urinary sodium excretion had fallen to 46 mmol. S-Na rose rapidly to 129 mmol/l one day after the change and 133 mmol/l two days later. Thereafter she has used digoxin, hydrochlorothiazide and potassium supplementation.

Case 2

A 68-year-old female had been examined earlier for epigastric pains. A decreasing aortic diastolic murmur had been heard and she had been digested. In Jan. 1979 she attended a health center for epigastric pains. She had extrasystoles with a BP of 00/95. The daily digoxin dose was halved and she received 1 tablet Durex (5 mg amiloride + 50 mg hydrochlorothiazide, Orion, Finland) daily.

During the following two weeks she suffered abdominal colic, palpitations, vertigo and dizziness and was admitted to the Central Hospital. After admission she was well at rest, there was epigastric tenderness and the BP was 180/80. B-Hb, B-leuc, s-transaminases and Astrup values were within normal limits. S-Na was 107 mmol/l although s-K, s-Ca, s-Pi and s-Mg were within normal limits. Durex was discontinued and she received sodium infusions for the following two days. S-Na rose gradually to 134 mmol/l within a week and she was discharged asymptomatic on 0.25 mg digoxin daily. Three months

later when she was in another hospital for epigastric discomfort, gallbladder, gastric erosion and kidney examination were within normal limits.

The patient was re-examined in Aug. 1979 (Medamor[®] MSD) 5 mg daily increased her daily urinary sodium excretion from 16 to 179 mmol and s-Na from 142 to 133 mmol/l within three days. Within a week it was back to 139 mmol/l before admission. She was discontinued. During the following weeks her s-Na remained at 130/95 and there was slight pulmonary congestion which led to treatment with 25 mg hydrochlorothiazide + 13 mmol potassium daily. By Feb. 1980 she felt well and s-Na remained above 135 mmol/l. Two weeks later the potassium supplementation was changed to 5 mg amiloride. The daily sodium excretion was 176 mmol before the change and 133 mmol two days after. The corresponding potassium excretions were 105 and 34 mmol. The only change in serum electrolytes was s-Na decrease to 118 mmol/l within one week. Amiloride was changed back to potassium supplementation. Three weeks later s-Na was 137 mmol/l where it has remained during continuing supplemental treatment with 5 mg hydrochlorothiazide + 13 mmol potassium daily.

Case 3

An 86-year-old female had used digoxin 0.25 mg, propranolol 300 mg and earlier hydrochlorothiazide 50 mg daily for years but in recent months 25 mg hydrochlorothiazide + 6.5 mmol potassium combination had been the daily diuretic. She was admitted to the Central Hospital following two days of vomiting and her symptoms cleared two days after withdrawal of the digoxin treatment and the institution of saline and potassium supplementation.

On admission B-gluc was 131 mmol/l, s-Na 131 mmol/l and s-K 3.3 mmol/l. During the first two days s-Na remained between 140 and 143 mmol/l, potassium excretions and s-K rose gradually to 4.3 mmol/l. B-gluc remained between 10.0 and 15.7 mmol/l during the first weeks. Because of the high B-gluc values chlorpropamide was changed to glibenclamide 5 mg \times 3 on admission. Because of hypokalemia the diuretic was changed 10 days later to Durex 1 tablet daily. Digoxin treatment was continued in a dose of 0.125 mg daily. She was well but showed some signs of general arteriosclerosis.

After six days 25 mg amiloride + hydrochlorothiazide treatment s-Na was 140 mmol/l and s-K 3.8 mmol/l. Amiloride + thiazide administration was discontinued but on the following morning 24 hours after the last dose of the combination s-Na was 119 mmol/l and s-K 3.1 mmol/l. During the following three days there was 131 mmol isotonic saline whereafter s-Na rose gradually to 131 mmol/l. S-Na and s-K remained later within normal limits.

The patient was readmitted four weeks after her admission due to a thrombus of the left popliteal vein. She received heparin + marcuric therapy in addition to 25 mg amiloride (Medamor[®]) 5 mg daily. Wastes of 141 mmol s-Na decreased from 141 to 135 mmol/l while s-K rose from 4.1 to 5.3 mmol/l. The latter s-Na returned to 139 mmol/l and s-K remained between 5.0 and 4.8 mmol/l in spite of continued amiloride therapy. After one week

side was withdrawn after which s-Na and s-K have remained within normal limits. 3 months later she was admitted to the same hospital to heart failure, pulmonary congestion and hydrothorax. The symptoms and X-ray findings cleared with 1 week with hydrochlorothiazide 50 mg + potassium supplementation 26 mmol daily. Thereafter daily potassium supplementation was decreased to 13 mmol and has remained well and s-Na and s-K have stayed within normal limits.

DISCUSSION

Three patients developed severe hyponatremia on amiloride + hydrochlorothiazide treatment. All tolerated hydrochlorothiazide treatment and potassium supplementation. Case 1 developed hyponatremia after several years' use and in the maintenance period hyponatremia developed on potassium supplementation was changed to furosemide. Case 2 developed hyponatremia during the first period of amiloride + hydrochlorothiazide. On follow-up she developed hyponatremia on as low a daily dosage as 25 mg hydrochlorothiazide + 13 mmol amiloride. Case 3 had used hydrochlorothiazide with potassium for years but she developed hyponatremia rapidly with the combination of amiloride + hydrochlorothiazide. There were no signs of thoracic infections or tumours which sometimes cause hyponatremia (11). Neither was there cardiac failure or edema, thus excluding dilutional hyponatremia. Normal plasma cortisol values and thyroid function tests excluded hormonal causes of hyponatremia and normal renal function excluded renal causes. There was no hypernatremia or such variations in B-glucose that could cause pseudohyponatremia.

One of the patients was hypokalemic during the hyponatremia period on the first hospital admission but she had vomited and seemed to be hypokalemic due to loss of potassium rich gastric contents. Hypokalemia does not seem to have been associated with the development of amiloride-induced hyponatremia. Most of the 25 diuretic hyponatremic patients presented by Fichman et al (1) were hypokalemic, only two having s-K levels >3.4 mmol/l. Four patients had hyponatremia corrected on massive potassium supplementation (200–240 mmol/day). In our patients potassium in doses of 13–26 mmol/day kept s-K and s-Na values within normal limits during maintenance therapy with hydrochlorothiazide. Thus the role of potassium seems to differ

between our patients and the patients with diuretic hyponatremia (1).

The difference in potassium metabolism may be a sign of different mechanisms behind amiloride-induced hyponatremia and normal diuretic hyponatremia. Fichman et al (1) presented the following mechanism for the latter: A deficit of intracellular potassium induced by thiazide administration might allow the entry of sodium ions into the cell, which may be followed by extracellular volume contraction and consequent stimulation of ADH release by activation of volume receptors. ADH activity is then followed by water retention and hyponatremia. The improvement in diuretic hyponatremia during massive potassium supplementation despite continued thiazide in the patients of Fichman et al (1) suggests displacement of intracellular Na^+ by K^+ and movement of Na^+ back to the intracellular fluid, thus lessening the volume receptor stimuli for ADH release. Amiloride has an effect on the mechanism that exchanges sodium with potassium in the distal tubule: it has a natriuretic effect of its own and it also enhances the natriuretic action of thiazides (6). Hyponatremia due to amiloride + hydrochlorothiazide might be due to a direct effect of these two drugs on the distal nephrons without any mediating effect of hypokalemia and ADH activity. This is in line with the findings of Houdent et al (3) who proposed that hyponatremia due to amiloride + hydrochlorothiazide is caused by transient interstitial nephropathy.

What is the frequency of amiloride hyponatremia? A prospective study by Kohvakka et al (5) detected no hyponatremic cases among 40 patients in three months. Among 213 patients presented during an international workshop in 1976, one case developed hyponatremia during thiazide therapy when potassium supplementation had been replaced by amiloride (11). In retrospective studies hyponatremia has occasionally been reported during amiloride + hydrochlorothiazide treatment. Pinnock (7) and Polanska and Baron (8) presented three cases each, all elderly patients. Further details of these cases are lacking. Houdent et al (3) presented five patients with s-Na below 120 mmol/l, only one of whom showed s-K under 3.4 mmol/l. Roberts et al (9) reported four cases of hyponatremia, one of whom had been treated with the amiloride + hydrochlorothiazide combination. Kennedy et al (4) presented eight hyponatremic patients, some of whom had used potassium re-

taining diuretics. The literature suggests that amiloride hyponatremia is quite rare but it may in fact be even more common than normal diuretic hyponatremia.

Hyponatremia during diuretic treatment for cardiovascular diseases might be divided into three types: 1) Patients with severe impairment of cardiac function with water intoxication (12). Such patients exhibit signs of edema, pulmonary congestion or edema on chest X-ray and nowadays they usually receive diuretic treatment. The hyponatremia in patients in a state of sodium and water overload is a consequence of excessive water retention. The diuretic treatment is not the cause of the hyponatremia; before the diuretic era such patients would probably have died from heart failure. Treatment is based on accurate diagnosis (pulmonary emboli, infections or other factors aggravating the heart failure), adequate digitalization, proper use of diuretics, occasional careful use of corticosteroids or osmotic diuretics and particularly water restriction. (12). Combined use of hypertonic saline and i.v. injections of loop diuretics has been suggested quite recently (2). 2) Usual diuretic hyponatremia. Hyponatremia is usually associated with hypokalemia and ADH activity and the electrolyte disturbances are rapidly corrected by discontinuing the diuretic treatment (1-9). The patients with this type of hyponatremia probably do not need diuretic treatment. 3) Amiloride hyponatremia may be an example of a pure pharmacologic effect of drugs on the nephrons to induce hyponatremia. The patients seem to need diuretic therapy but it appears appropriate to continue it by changing amiloride to furosemide or bumetanide. Because the vague side effects and disorientation due to hyponatremia can easily be taken as a sign of a terminal stage of other diseases in elderly patients, amiloride hy-

ponatremia should be born in mind in the future. These cases remind us of the importance of monitoring serum Na values routinely when water measurements, particularly in patients with symptoms.

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Carditis and Arthritis Associated with *Campylobacter jejuni* Infection

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STRACT Three patients who developed carditis and enteritis caused by *Campylobacter jejuni* are reported. Two had perimyocarditis and one endocarditis. The cardiac sequelae persisted in two patients. Joint arthritis appeared in two of these patients: monoarthritis in one and polyarthritis in the other.

Keywords: *Campylobacter jejuni*, perimyocarditis, endocarditis, arthritis.

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Four cases of arthritis due to *Campylobacter fetus jejuni* (C. jejuni) have recently been described (4, 8). Carditis has been associated with enteritis due to *Shigella*, *Salmonella* and *Yersinia enterocolitica* (5, 6, 7) but to our knowledge not with enteritis due to C. jejuni. We report three patients: one with carditis and two with both carditis and arthritis. They were found among 342 patients with C. jejuni enteritis diagnosed at our laboratory in the end of 1979.

CASE REPORTS

Case 1
Previously healthy 25-year-old man fell ill with diarrhoea of two days' duration when visiting Cuba. A few days later migratory polyarthritis developed gradually affecting both knees and ankles, the left hip and the right shoulder. About two weeks after the onset of illness the patient was admitted to a hospital in Finland, where cardiac auscultation revealed both systolic and diastolic murmurs. The cardiologist diagnosed endocarditis affecting mitral and aortic valves.
The result of bacteriological examination of the stool after 7 days of antibiotic treatment one week after admission was positive for C. jejuni. Agglutination test against a fellow isolate of C. jejuni strain revealed a titre of 1:600 that fell to 1:100 one month later. Eight months later it

had fallen to 1:200. ECG and chest radiograph revealed no pathological features.

ESR was initially 119 mm/h, leucocyte count $13 \times 10^9/l$ and Hb 12.5 g/dl. In three weeks the Hb fell to 10.5 g/dl. Serum ASAT 0.80 $\mu\text{kat/l}$ and ALAT 1.38 $\mu\text{kat/l}$ were increased (normal 0.08-0.32). Tissue typing showed HLA A W19 B27.40 W4 W6 CW2.

The patient received first gentamicin and imipenem and later erythromycin. The fever subsided gradually within two weeks and the arthritis within four weeks and the patient was discharged after five weeks. At a check-up four months later echocardiography showed thick stiff leaflets of aortic valve and systolic and diastolic murmurs corresponding to mitral and aortic regurgitation were still audible. His working capacity remained subnormal and he was not able to return to his previous physical labour.

Case 2

A 32-year-old female cook was admitted with a 15-day history of diarrhoea. She had had congenital syphilis which had been treated with penicillin 11 years earlier and was regarded as fully cured. On the day before admission her right knee had become painful and swollen and a slight systolic murmur was heard at the aortic area and apex. The physical findings were otherwise normal.

C. jejuni was isolated from a stool specimen. However no agglutination against her own strain were found in the serum. Antibodies to *Treponema pallidum* were found in immobilization and haemagglutination tests but not in the complement fixation test. On admission slight elevation of S-T segment was seen in the ECG in leads V2-V4 and the P-Q interval was prolonged 0.23 sec, later 0.29 sec. ESR was initially 24 mm/h and the leucocyte count normal. Radiographs of the chest and right knee showed no pathological changes. Tissue typing showed HLA A2.9 B15 W6 W22 CW1 and CW3.

Without any specific treatment the diarrhoea subsided on the fourth and the arthritis on the eighth day of hospitalization. At a check-up ten months later the patient still complained of pain in her right knee on exertion but no objective changes could be detected in the joint. The systolic murmur was still audible and the P-Q interval prolonged 0.24 sec.

Case 3

A 27-year-old man was admitted with febrile diarrhoea of eight days' duration which had its onset in Greece. On

day before admission he had experienced severe pain across the front of his chest radiating to the left upper limb. Examination of the heart and chest showed nothing abnormal.

On admission elevation of S-T segment and inversion of T waves were seen in leads I, II, aVF and V4-V6. ESR was initially 24 mm/h and the leucocyte count normal. Serum LDH was 8.93 μ kat/l (normal 3.33-7.50), this increase was found to be due to the first isoenzyme fraction. The continuous chest pain, like the fever and diarrhoea, subsided without treatment within the first day of hospitalization and the exertional chest pain disappeared gradually within two weeks. However the ECG changes were still present after a further two weeks. Two months later the patient was in good health and the ECG had normalized.

Stool culture yielded growth of *C. jejuni* on admission and five weeks after the disappearance of diarrhoea; subsequently the culture remained negative. The agglutinating antibody titre for his own *C. jejuni* strain was 1:800 when measured three and five weeks after the onset of illness. Three months later the titre was 1:50. Tissue typing showed HLA A 3 W19 B 8 B 18 W 6 C.

In all three patients serological tests were negative for rheumatoid factor and antistreptolysin O, as well as for antinuclear, *Y. enterocolitica* and *Neisseria gonorrhoea* antibodies. No rises in titre were observed in the Widal test. Stool culture for *Salmonella*, *Shigella* and *Y. enterocolitica* showed no growth. No symptoms of genitourinary or respiratory tract infection were observed.

DISCUSSION

The present findings suggest that *C. jejuni*, like *Salmonella*, *Shigella* and *Y. enterocolitica*, may cause both carditis and arthritis after enteritis. The aetiological diagnosis was established in one case by cultivating the organism from stool, in the second by detecting high antibody titres and in the third by both these methods. No alternative causative organism or factor for carditis was detected in cases 1 and 3. Whether the cardiac involvement in case 2 was due to *C. jejuni* infection or to non-specific exacerbation of possible earlier damage caused by congenital syphilis could not be decided with certainty.

Carditis, both endocarditis and perimyocarditis, is now reported for the first time in association with *C. jejuni* infection. This organism is known to be able to cause bacteraemia, thus it is not surprising

that it like *Salmonella* can cause endocarditis and perimyocardial involvement in the two other patients as well as the interval of 1-2 weeks between the onset of intestinal and cardiac symptoms suggest that carditis due to *C. jejuni* may also be reactive in nature.

Arthritis has been reported as a rare manifestation of *C. jejuni* infection. The patients previously reported in the literature have all had oligoarthritis. The findings in our case 2 suggest that *C. jejuni* may also cause monoarthritis.

Arthritis after *Salmonella* and *Y. enterocolitica* infections is known to be especially frequent in patients with HLA B 27 (1-3). Of the previously tested three patients with arthritis due to *C. jejuni* one was positive for this histocompatibility antigen and now one of the two patients with arthritis. Thus it is possible that positivity for HLA B 27 also predisposes to arthritis due to *C. jejuni*.

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SUPPLEMENTS TO VOLUME 208

641 Arrhythmias and other cardiovascular responses during Finnish sauna and exercise testing in healthy men and post myocardial infarction patients By O J Luunila

642 Thrombosis and blood vessel wall interactions in coronary heart disease Proceedings of the fifth Paavo Nurmi symposium Porvoo Finland September 20-22 1979 Edited by V Manninen C Wood and J I Halonen

Interaction between blood cells and the vessel wall—An overview By E Ikkala The anti thrombotic effects of prostacyclin By J R Vane and S Moncada One two three or more pathways for platelet aggregation By B B Vargafing M Chignard J P Le Couedic and J Benveniste A new mechanism for the regulation of platelet functional state By R A Markosyan Effect of various agents on prostaglandin biosynthesis and the anti aggregatory effect By W Forster In vivo and ex vivo studies of the effect of vitamin E pre treatment on PGI₂ and TXA₂ synthesis By W Forster Platelets smooth muscle proliferation and atherosclerosis By R Ross Serum lipoprotein composition platelet factor and arterial smooth muscle cells By T Ronnema Prostaglandins and cyclic nucleotides in hypoxic myocardium By H Vapaatalo T Metsä Ketela and K Laustiola Platelet taurine in patients with arterial hypertension myocardial failure or infarction By M K Paasonen O Penttilä J J Himberg and E Solatunturi β Thromboglobulin in acute myocardial infarction By V Rasi I Torstila and E Ikkala Morphological variants of acute myocardial necrosis and their relationship to coronary artery thrombosis By M Woolf and M J Davies Platelets intravascular coagulation and fibrinolysis in hyperlipidaemias Relationship to thrombo-embolic complications By A C A Carvalho and R S Lees The interactions of lipids platelets and endothelial cells in thrombogenesis By A Nordøy Resistance to atherosclerosis in pigs with von Willebrand's disease By E J Bowie and V Fuster The thrombo haemorrhagic balance By H Stormorken Blood coagulation in relation to coronary heart disease By J A Davies Patterns of repair in the arterial wall and their possible relationship to growth characteristics of smooth muscle and endothelium By S Björkerud Characterization of foam cells in experimental atherosclerosis By S Fowler Factors modulating the interaction of LDL with an arterial lipoprotein complexing proteoglycan the effect of HDL By G Camejo M M Cortez F Lopez R Starosta B Mosquera and L Socorro Fibronectin and atherosclerosis By S Stenman K von Smitten and A Vaheri Modulation of prostaglandin production in tissues by dietary essential fatty acids By C Galli E Agradi A Petroni and A Socini Non steroidal anti inflammatory agents and coronary heart disease By J F Mustard M A Packham and R L Kinlough Rathbone Possible approaches to the pharmacological prevention of myocardial ischaemia By G V R Born and A Wehmeier General Discussion

643 On the metabolism of cholesterol and bile acids in normo- and hyperlipidaemic subjects By E Andersen

SUBJECT INDEX

(Supplements see p V)

Adrenals

- Preoperative localization of aldosterone producing adenomas (Adamson, Efendić, Granberg, Lindvall, Lins & Low) 101
- Cortisone induced remission of hypothyroidism in Schmidt's syndrome (Draminsky, Petersen & Bergman) 125

Alcohol

- Serum and urinary myoglobin in alcoholics (Hallgren, Lundin, Roxin & Venge) 33

Amyloidosis

- Atrioventricular and intraventricular conduction in familial amyloidosis with polyneuropathy (Olofsson, Andersson & Furberg) 77
- Amyloid deposits in bone marrow aspirates in primary amyloidosis (Stavem, Frøyskov, Larsen, Ly & Rørvik) 111
- Demonstration and partial characterization of an atypical protein in the urine of a patient with primary amyloidosis (Wille, Olsen, Førre, Sletten & Jentoft) 177

Anaemia

- Castle's test (with vitamin B₁₂ and normal gastric juice) in the ileum in patients with genuine and patients with tapeworm pernicious anaemia (von Bonsdorff & Gårdnér) 193

Anticoagulants

- The outcome of patients with transient ischaemic attacks and stroke treated with anticoagulants (Terent & Andersson) 39

Arrhythmia

- Antiarrhythmic properties of a neuroleptic butyrophenone, melperone, in acute myocardial infarction (Mugelvang, Petersen, Folke & Ovesen) 61
- Ventricular arrhythmias during exercise testing and 24-hour ECG tape recording in patients with ischaemic heart disease and in normal individuals (Møller & Thygesen) 65
- Episodic cardiac arrhythmia and accident rate (Nilsson & Abdon) 69
- Episodic cardiac arrhythmia and femoral neck fracture (Abdon & Nilsson) 73
- Sinus node dysfunction in 18 patients (Sjomsen, Strøde, Nielsen & Løjsgaard Nielsen) 343
- Prodromal ventricular premature beats preceded by a diastolic wave (Ejvinsson & Ornust) 443

Arteries

- Coronary ischaemia and occlusion in giant cell (temporal) arteritis (Paulley) 257
- Gangrene localized in the lower limbs in diabetes (Lithner & Tornblom) 313

Biopsy

- Amyloid deposits in bone marrow aspirates in primary amyloidosis (Stavem, Frøyskov, Larsen, Ly & Rørvik) 111
- Studies on hemopoietic dysplasia in the preleukemic syndrome (Ludbeck) 49

Blockers

- The effect of beta blockade on glucose tolerance and insulin release in adult diabetes (Holm Johansson Vedin Wilhelmsson & Smith) 187

Blood

- Plasmapheresis—*Bloodletting* revived and refined (Waldenström) 1
 On acquired hemophilia A (Nilsson & Lammé) 5
 Effect of long term treatment with human leukocyte interferon on various laboratory parameters (Ingemarsson Bergström Broström Cantell & Strander) 155
 Castle's test (with vitamin B₁₂ and normal gastric juice) *in vivo* in patients with genuine and patients with tapeworm pernicious anaemia (von Bonsdorff & Gordin) 193
 Studies on hemopoietic dysplasia (the preleukemic syndrome) (Lidbeck) 459

Blood pressure

- Blood pressure and renal function (Ljungman Aurell Hartford Wikstrand Wilhelmsson & Berglund) 37
 Comparison of energy and nutrient intakes in women with high and low blood pressure levels (Thulin Abdulla, Dencker Jägerstad Melander Norden Scherstién & Åkesson) 367
 Blood pressure control in a middle aged male population (Åberg Hedstrand & Lithell) 467

Bone

- Episodic cardiac arrhythmia and femoral neck fracture (Abdon & Nilsson) 73
 Amyloid deposits in bone marrow aspirates in primary amyloidosis (Stavem Frøyshov Larsen Ly & Rørvik) 311
 Osteoclastic myeloma with polyneuropathy (Reitan Pape Foså Jølsrud Stetnes & Solheim) 137
 Elevated bone phosphorus/hydroxyproline ratio following jejunocolic bypass surgery (Hey & Tougaard) 321

Brain

- Pseudotumor cerebri in pseudohypoparathyroidism (Asplund) 331
 Hypoglycemia in Alzheimer's disease (Adolfsson Bucht Lithner & Wirtblad) 387

Calcium

- A case of hypocalcemia heart failure and exceptional repolarization disturbances (Murois & Luomanmäki) 133

Cancer

- Urinary excretion of inorganic sulfate ester sulfate total sulfur and taurine in cancer patients (Baldertorp & Mårtensson) 293
 Multiple myeloma and gastric carcinoma (Peters & Mackay) 411

Cerebrovascular disease

- A study of stroke patients treated in a non intensive stroke unit or in general medical wards (von Arbin Britton de Faire Helmers Miah & Murray) 81

Circulation

- Aneurysm of the popliteal vein as a cause of pulmonary embolism (Persson Donner Petersson Eklof & Wintzell)

Coagulation

On acquired hemophilia A (Nilsson & Lammé)

5

Collagen disease

Multifocal idiopathic fibrosclerosis (Kraemmer Nielsen)

119

Cytology

Abnormal pattern of the rough endoplasmic reticulum of plasma cells in multiple myeloma with multiple concentric lamellar bodies and "single sac loops" (Stavem Ly & Rørvik)

115

Structure and function of neutrophil leukocytes from patients with the immotile-cilia syndrome (Afzelius Ewetz, Palmblad Liden & Ventzelos)

145

Diabetes mellitus

Symptomatic diabetes mellitus cured by potassium and withdrawal of polythiazide in a hypokalemic hypertensive woman (Nilsson)

179

The effect of beta blockade on glucose tolerance and insulin release in adult diabetes (Holm Johansson Vedin Wilhelmsson & Smith)

187

Excessive sensitivity to the hyponatremic effect of chlorpropamide in a patient with diabetes mellitus and anterior pituitary insufficiency (Aasen & Frey)

233

Blood glucose control and lipolysis in diabetes mellitus (Arner Engfeldt & Östman)

297

Comparative single-dose kinetics and effects of four sulfonylureas in healthy volunteers (Sartor Melander Schersten & Wahlén Boll)

201

Early insulin response in latent gestational diabetes (Blöhmé Karlsson & Waldenström)

309

Gangrene localized to the lower limbs in diabetes (Lutthner & Tornblom)

315

Effect of different kinds of fibre on postprandial blood glucose in insulin-dependent diabetics (Væder Hansen & Aagaard)

339

24-Hour blood glucose profiles in insulin-dependent diabetics treated with an ravenous insulin infusion systems (Decker Boysen Sandahl Christiansen Holendorf Aaby Svendsen & Andersen)

441

Diagnosis

Serum and urinary myoglobin in alcoholics (Hallgren Lundin Ruxin & Venge)

33

Preoperative localization of aldosterone producing adenomas (Adamson Esfendi Granberg Lindvall Lins & Low)

101

Electrocardiographic diagnosis of ventricular septal infarction (Hellerstedt Jonasson & Örnäs)

213

Thallium-201 scintigraphy after acute myocardial infarction (Bone Carlens Holmgren Johnson Jonasson Kandilas Mogensen Nordlander & Örnäs)

219

Enzyme activities in serum after extensive exercise with special reference to creatine kinase MB (Schrohr Grande & Christiansen)

229

Skeletal scintigraphy with technetium diphosphonate in multiple myeloma—a comparison with skeletal X ray (Lindström & Lindström)

287

Urinary excretion of inorganic sulfate ester sulfate total sulfur and taurine in cancer patients (Baldertorp & Martensson)

293

Pseudotumor cerebri in pseudohypoparathyroidism (Asplund)

331

The clinical impact of long term ECG recording (Eriksson & Pahlm)

345

Flexible fiberoptic bronchoscopy in sarcoidosis (Stjernberg Björnsdahl Pettersen & Truedsson)

347

Diuretics

Urinary zinc excretion during treatment with different diuretics (Westert)

259

Zinc balance before and during treatment with bendroflumethiazide (Westert)

265

Tissue zinc at autopsy—Relation to medication with diuretics (Westert)

263

- Effects of mefruside treatment in hypertension (Henningssen Bergengren Malmberg Pihl Ren marker & Strand) 273
- Increased urinary protein excretion after intravenous injection of furosemide in man (Ylitalo Pasternack Kallio Vantinen & Metsä Ketela) 279

ECG

- QT intervals at discharge after acute myocardial infarction and long term prognosis (Ahneve Helfmers & Lundman) 55
- Ventricular arrhythmias during exercise testing and 24-hour ECG tape recording in patients with ischaemic heart disease and in normal individuals (Møller & Thaysen) 65
- Atrioventricular and intraventricular conduction in familial amyloidosis with polyneuropathy (Olofsson Andersson & Furberg) 77
- A case of hypocalcemia heart failure and exceptional repolarization disturbances (Murros & Luomannaki) 133
- Electrocardiographic diagnosis of ventricular septal infarction (Hellerstedt Jonasson & Ornnius) 213
- Effect of metoprolol on QT intervals after acute myocardial infarction (Ahneve Erhardt Lund man Rehnqvist & Sjogren) 223
- Sinus node dysfunction in 128 patients (Simonsen Stræde Nielsen & Lyager Nielsen) 343
- The clinical impact of long term ECG recording (Erksson & Pahlm) 355
- Prodromal ventricular premature beats preceded by a diastolic wave (Ejvinsson & Ornnius) 445
- Sinus node dysfunction in acute myocardial infarction (Simonsen Lyager Nielsen & Stræde Nielsen) 463

Electrolytes

- Symptomatic diabetes mellitus cured by potassium and withdrawal of polythiazide in a hypokalemic hypertensive woman (Nilsson) 129
- Urinary zinc excretion during treatment with different diuretics (Wester) 209
- Excessive sensitivity to the hyponatremic effect of chlorpropamide in a patient with diabetes mellitus and anterior pituitary insufficiency (Aasen & Frey) 233
- Urinary excretion of inorganic sulfate ester sulfate total sulfur and taurine in cancer patients (Baldetorp & Mårtensson) 293
- Elevated bone phosphorus/hydroxyproline ratio following jejunoileal bypass surgery (Hey & Lougaard) 321
- Amiloride induced hyponatremia (Tarssanen Huikko & Rossi) 491

Electron microscopy

- Abnormal pattern of the rough endoplasmic reticulum of plasma cells in multiple myeloma with multiple concentric lamellar bodies and single sac loops (Stavem Ly & Rørvik) 115
- Membranous glomerulopathy in a patient on captopril (Hoorntje Donker Prins & Weening) 325

Endoscopy

- Flexible fiberoptic bronchoscopy in sarcoidosis (Sjoberg Bjornstad Pettersen & Truedsson) 397

Enzymes

- Enzyme activities in serum after extensive exercise with special reference to creatine kinase MB (Schnohr Grande & Christiansen) 229
- Angiotensin-converting enzyme in newly detected sarcoidosis (Rømer) 437

Epidemiology

- The epidemiology of thyrotoxicosis in Denmark (Mogensen & Green)

Exercise

- Ventricular arrhythmias during exercise testing and 24-hour ECG tape recording in patients with ischaemic heart disease and in normal individuals (Møller & Thygesen) 65
- Enzyme activities in serum after extensive exercise with special reference to creatine kinase MB (Schnohr, Grande & Christiansen) 229

Fatty acids

- Dietary fatty acids and ischaemic heart disease (Hamilton, Lea & Jones) 137

Gastrointestinal tract

- The effects of cimetidine (Tagamet®) on renal function in patients with renal failure (Larsson, Bodemar, Hågedal & Wadén) 27
- Report on a patient with watery diarrhoea syndrome caused by a pancreatic tumour containing neurotensin, enkephalin and calcitonin (Gutniak, Rosenqvist, Grmelius, Lundberg, Hökfelt, Rokaeus, Rosell, Lundqvist, Fahrenkrug, Sundblad & Gutniak) 95
- Castle II test (with vitamin B₁₂ and normal gastric juice) in the ileum in patients with genuine and patients with tapeworm pernicious anaemia (von Bonsdorff & Gordin) 193
- Elevated bone phosphorus/hydroxyproline ratio following jejunioileal bypass surgery (Høy & Tougaard) 111
- Carditis and arthritis associated with *Campylobacter jejuni* infection (Punka, Pitkanen, Pettersson, Aittoniemi & Kosunen) 495

Geriatrics

- Vitamin D deficiency in welfare institutions for the aged (Toss, Almqvist, Larsson & Zetterqvist) 87

Glucose

- The effect of beta blockade on glucose tolerance and insulin release in adult diabetes (Holm, Johansson, Vedin, Wilhelmsson & Smith) 187
- Blood glucose control and lipolysis in diabetes mellitus (Arner, Engfeldt & Östman) 297
- Comparative single-dose kinetics and effects of four sulfonylureas in healthy volunteers (Sartor, Melander, Schersten & Wahlén) 301
- Effect of verapamil on blood glucose and serum insulin in patients with hyper- and hypothyroidism (Andersson & Rydmark) 375
- Hypoglycemia in Alzheimer's disease (Adolfsson, Bucht, Lathner & Winblad) 387
- Effect of different kinds of fibre on postprandial blood glucose in insulin-dependent diabetes (Vælder Hansen & Aagaard) 387
- 24-Hour blood glucose profiles in insulin-dependent diabetics treated with intravenous insulin infusion systems (Deckert, Boysen, Sandahl, Christiansen, Juulendorf, Aaby, Svendsen & Andersen) 481

Heart

- Atrioventricular and intra-ventricular conduction in familial amyloidosis with polyneuropathy (Olsson, Andersson & Furberg) 22
- A case of hypocalcemia, heart failure and exceptional repolarization disturbances (Morton & Luomanmaki) 133
- Coronary ischaemia and occlusion in giant cell (temporal) arteritis (Pauzay) 257
- Dietary fatty acids and ischaemic heart disease (Hamilton, Lea & Jones) 137
- Sinus node dysfunction in 128 patients (Simonsen, Strade, Nielsen & Løjsgaard) 243
- The diagnosis of aural myxomas (Schlossman, Selin, Wallin & Wallentin) 327
- Carditis and arthritis associated with *Campylobacter jejuni* infection (Punka, Pitkanen, Pettersson, Aittoniemi & Kosunen) 495

Hemophilia

- On acquired hemophilia A (Nilsson & Lammé) 5

Heredity

- Structure and function of neutrophil leukocytes from patients with the *immotile cilia* syndrome (Afzelius Ewert Palmblad Uden & Venizelos) 145
- High immune responsiveness in a family with multiple paraproteinemia and autoimmune thyroid disease (Weits de Gast The van der Giesen Ockhuizen Festen & Mandema) 169

Hormones

- Serum reverse T_3 determinations in the laboratory diagnosis of hyperthyroidism (Ljunggren & Kallner) 91
- Report on a patient with watery diarrhoea syndrome caused by a pancreatic tumour containing neurotensin enkephalin and calcitonin (Gutniak Rosenqvist Grimelius Lundberg Høkdelt Rokaeus Rosell Lundqvist Fahrenkrug Sundblad & Gutniak) 95
- Preoperative localization of aldosterone producing adenomas (Adamson Efendić Granberg Lindvall Lins & Low) 101
- Excessive sensitivity to the hyponatremic effect of chlorpropamide in a patient with diabetes mellitus and anterior pituitary insufficiency (Aasen & Frey) 233
- Pseudotumor cerebri in pseudohypoparathyroidism (Asplund) 331

Hypertension

- Hydralazine in arterial hypertension (Wulff Lenz Krogsgaard & Holst) 49
- Symptomatic diabetes cured by potassium and withdrawal of polythiazide in a hypokalemic hypertensive woman (Nilsson) 129
- Effects of mefruside treatment in hypertension (Henningsen Bergengren Malmberg Pihl Ren marker & Strand) 273
- Membranous glomerulopathy in a patient on captopril (Hoorntje Donker Prins & Weening) 325
- Emergency treatment of severe hypertension evaluated in a randomized study (Danish Multicenter Study) 473
- Borderline hypertension (Julius Hansson Andren Guldbrandsson Svartesson & Siennson) 481

Immunoglobulins

- High immune responsiveness in a family with multiple paraproteinemia and autoimmune thyroid disease (Weits de Gast The van der Giesen Ockhuizen Festen & Mandema) 169
- Occurrence of adult Fanconi syndrome in benign monoclonal gammopathy (Dahlstrom Mårtensson & Lindstrom) 425

Immunology

- Immunological studies on human thymus (Christensson Matell & Biberfeld) 161
- High immune responsiveness in a family with multiple paraproteinemia and autoimmune thyroid disease (Weits de Gast The van der Giesen Ockhuizen Festen & Mandema) 169
- Predominant B lymphocyte deficiency in a case with lymph node disease resembling angioimmunoblastic lymphadenopathy (Persson Rausing Turesson & Zettervall) 237

Infection

- Bacteriuria in a population sample of women (Bengtsson Bengtsson & Lincoln) 417
- Lymph node toxoplasmosis (Miettinen Saxon & Saxon) 431
- Carditis and arthritis associated with *Campylobacter jejuni* infection (Ponka Pitkanen Pettersson Aittoniemi & Kosunen) 495

Insulin

- The effect of beta blockade on glucose tolerance and insulin release in adult diabetes (Hilim Johansson, Vedin, Wilhelmsson & Smith) 187
- Early insulin response in latent gestational diabetes (Blohmé, Karlsson & Waldenström) 207
- Effect of verapamil on blood glucose and serum insulin in patients with hyper- and hypothyroidism (Andersson & Røydmark) 3-5
- 24 Hour blood glucose profiles in insulin-dependent diabetics treated with intravenous insulin infusion systems (Decker, Boysen, Sandahl, Christiansen, Holendorf, Aaby, Svendsen & Andersen) 451

Intoxication

- Amiloride induced hyponatremia (Tarssanen, Huikko & Rossi) 491

Isotopes

- Skeletal scintigraphy with technetium diphosphonate in multiple myeloma—a comparison with skeletal X ray (Lindström & Lindström) 259

Kidney

- Blood pressure and renal function (Ljungman, Aurell, Hartford, Wikstrand, Wilhelmson & Berglund) 17
- The effects of cimetidine (Tagamet®) on renal function in patients with renal failure (Larsson, Bodemar, Kågedal & Walan) 27
- Multifocal idiopathic fibrosclerosis (Krammer, Nielsen) 119
- Psychological and social problems encountered in active treatment of chronic uraemia III (Hirvas, Enckell, Kuhlback & Pasternack) 283
- Membranous glomerulopathy in a patient on captopril (Hoornijde, Donker, Prins & Weening) 325
- Renal function and morphology in long term lithium and combined lithium neuroleptic treatment (Bucht, Wahlén, Wentzel & Winblad) 381
- Occurrence of adult Fanconi syndrome in benign monoclonal gammopathy (Dahlström, Mårtensson & Lindström) 425
- Angiotensin-converting enzyme in newly detected sarcoidosis (Rømer) 437

Leukemia

- Lymphocytopenia preceding chronic lymphocytic leukemia (Brandt & Nilsson) 15
- Studies on hemopoietic dysplasia (the preleukemic syndrome) (Lidbeck) 459

Leukocytes

- Structure and function of neutrophil leukocytes from patients with the immotile-cilia syndrome (Mzeli, Lwetz, Palmblad, Udén & Venizelos) 145
- Effect of long term treatment with human leukocyte interferon on various laboratory parameters (Ingimarsson, Bergström, Broström, Cantell & Strandén) 155

Lipids

- Serum lipids with special reference to HDL cholesterol and triglycerides in young male survivors of acute myocardial infarction (Kaukola, Manninen & Halonen) 41
- Effect of guar gum on body weight and serum lipids in hypercholesterolemic females (Tuomilehto, Voutilainen, Huttunen, Vainio & Hämäläinen) 45
- The relation between the levels of HDL cholesterol and the capacity for removal of triglycerides (Sauer, Skrede, Enkassen & Blomhoff) 159

Blood glucose control and lipolysis in diabetes mellitus (Arner, Engvald & Östman)	297
Dietary fatty acids and atherosclerotic heart disease (Hamilton, Lea & Sjöberg)	337
The lipid hypothesis (Tyrö)	341
Lipoproteins	
Serum lipids with special reference to HDL cholesterol and triglycerides in young male subjects	41
Use of the myocardial ischaemia (Hakola, Manninen & Hakola)	79
HDL increasing effect of fish oil (Persson & Fex)	
Liver	
Giant cell carcinoma in a patient receiving carbamazepine (Levander)	153
Lung	
Aneurysm of the popliteal artery as a cause of pulmonary embolism (Persson, Österberg & Eriksson)	277
Effect of 4% nifedipine	
Lymph nodes	
Lymph node toxoplasmosis (Wittchen, Sørensen & Sørensen)	471
Lymphocytes	
Lymphocytopenia preceding chronic lymphocytic leukemia (Brundt & Vabnick)	13
Predominant B lymphocyte deficiency in a case with lymph node disease resembling a lymphoma (Persson, Rasmussen, Turesson & Zettervall)	237
Lymphoma	
Predominant B lymphocyte deficiency in a case with lymph node disease resembling a lymphoma (Persson, Rasmussen, Turesson & Zettervall)	237
Malformation	
Structure and function of neutrophil leukocytes from patients with the unimpaired-cilia syndrome (Afzelius, Eweltz, Palmblad, Ulfvén & Venzel)	345
Metabolism	
The relation between the levels of HDL cholesterol and the capacity for removal of triglycerides (Sawar, Skrede, Enkssen & Blomhoff)	199
Hypoglycemia in Alzheimer's disease (Adolfsson, Bucht, Lithner & Wimbö)	307
Occurrence of adult Fanconi syndrome in benign monoclonal gammopathy (Dahlström, Mårtensson & Linder)	425
Muscles	
Serum and urinary myoglobin in alcoholics (Hallgren, Lundin, Rönner & Venge)	33
Myeloma	
Abnormal pattern of the rough endoplasmic reticulum of plasma cells in multiple myeloma with multiple concentric lamellar bodies and spherule loops (Ståhl, Ly & Rönner)	115
Osteosclerotic myeloma with polyneuropathy (Reitan, Pape, Fosha, Jørgensen, Sletten & Solheim)	137

Insulin

- The effect of beta blockade on glucose tolerance and insulin release in adult diabetes (Holm Johansson Vedin Wilhelmsson & Smith) 187
- Early insulin response in latent gestational diabetes (Blohmé Karlsson & Waldenström) 309
- Effect of verapamil on blood glucose and serum insulin in patients with hyper- and hypothyroidism (Andersson & Rojdmärk) 375
- 24-Hour blood glucose profiles in insulin-dependent diabetics treated with intravenous insulin infusion systems (Deckert Boysen Sandahl Christiansen Holendorf Aaby Svendsen & Andersen) 451

Intoxication

- Amiloride induced hyponatremia (Tarssanen Huikko & Rossi) 491

Isotopes

- Skeletal scintigraphy with technetium diphosphonate in multiple myeloma—a comparison with skeletal X ray (Lindström & Lindström) 289

Kidney

- Blood pressure and renal function (Ljungman Aurell Hartford Wikstrand Wilhelmsson & Berglund) 17
- The effects of cimetidine (Tagamet®) on renal function in patients with renal failure (Larsson Bodemar Häggedal & Wälan) 27
- Multifocal idiopathic fibrosclerosis (Kremmer Nielsen) 119
- Psychological and social problems encountered in active treatment of chronic uraemia III (Hirvas Enckell Kuhlback & Pasternack) 285
- Membranous glomerulopathy in a patient on captopril (Hoomiya Donker Prins & Weening) 325
- Renal function and morphology in long term lithium and combined lithium-neuroleptic treatment (Bucht Wahlén Wentze & Winblad) 381
- Occurrence of adult Fanconi syndrome in benign monoclonal gammopathy (Dahlström Wärtenson & Lindström) 425
- Angiotensin-converting enzyme in newly detected sarcoidosis (Romer) 437

Leukemia

- Lymphocytopenia preceding chronic lymphocytic leukemia (Brandt & Nilsson) 13
- Studies on hemopoietic dysplasia (the preleukemic syndrome) (Lidbeck) 459

Leukocytes

- Structure and function of neutrophil leukocytes from patients with the immotile-cilia syndrome (Afzelius Ewertz Palmblad Uden & Venizelos) 145
- Effect of long term treatment with human leukocyte interferon on various laboratory parameters (Ingemarsson Bergström Broström Cantell & Strander) 155

Lipids

- Serum lipids with special reference to HDL cholesterol and triglycerides in young male survivors of acute myocardial infarction (Kaukola Mäkinen & Halonen) 41
- Effect of guar gum on body weight and serum lipids in hypercholesterolemic females (Tuomilehto Voutilainen Hutunen Vinni & Homan) 45
- The relation between the levels of HDL cholesterol and the capacity for removal of triglycerides (Sauer Skrede Erikssen & Blomhoff) 199

Bacteriuria in a population sample of women (Bengtsson Bengtsson & Lincöln)	417
Blood pressure control in a middle aged male population (Åberg Hedstrand & Lithell)	467
Borderline hypertension (Julius Hansson Andren Guldbrandsson Sivertsson & Svensson)	481

Pregnancy

Early insulin response in latent gestational diabetes (Blohme Karlsson & Waldenström)	309
---	-----

Prognosis

QT intervals at discharge after acute myocardial infarction and long term prognosis (Ahne Helmers & Lundman)	55
A study of stroke patients treated in a non intensive stroke unit or in general medical wards (von Arbin Britton de Faire Helmers Mah & Murray)	81

Proteins

High immune responsiveness in a family with multiple paraproteinemia and autoimmune thyroid disease (Weill de Gaudy The van der Gessen Ockhuizen Festen & Mandema)	169
Demonstration and partial characterization of an atypical protein in the urine of a patient with primary amyloidosis (Wille Olsen Førre Sletten & Jentoft)	177
Increased urinary protein excretion after intravenous injection of furosemide in man (Ylitalo Pasternack Hallö Vanittanen & Metsä-Ketelä)	279
Circulating protein complexes in D-penicillamine therapy of rheumatoid arthritis (Norberg Wollheim & Gedda)	393

Psychiatry

Renal function and morphology in long term lithium and combined lithium neuroleptic treatment (Bucht Wahlén Wentzel & Wänblad)	381
Hypoglycemia in Alzheimer's disease (Adolfsson Bucht Lithner & Wänblad)	387

Psychology

Psychological and social problems encountered in active treatment of chronic uraemia III (Hrvas Enckell Kuhiback & Pasternack)	285
--	-----

Rheumatoid arthritis

Circulating protein complexes in D-penicillamine therapy of rheumatoid arthritis (Norberg Wollheim & Gedda)	393
---	-----

Sarcoidosis

Fibre broncho copy in sarcoidosis (Sjoberg Bjornstad Jørgensen & Truedson)	397
Angiotensin converting enzyme in newly detected sarcoidosis (Rømer)	437

Skeleton

Episodic cardiac arrhythmia and femoral neck fracture (Abdon & Nilsson)	73
Osteosclerotic myeloma with polyneuropathy (Reitan Pape Fosså Julsrud Sletten & Solheim)	137
Skeletal scintigraphy with technetium diphosphonate in multiple myeloma—a comparison with skeletal X-ray (Lindström & Lindström)	289
Elevated bone phosphorus/hydroxyproline ratio following jejunoileal bypass surgery (Hey & Tougaard)	

Smoking

- Smoking habits in the Glostrup population of men and women born in 1914 (Schroll) 745

Stroke

- A study of stroke patients treated in a non intensive stroke unit or in general medical wards (von Arbin Britton de Faire Helmers Miah & Murray) III
- The outcome of patients with transient ischemic attacks and stroke treated with anticoagulants (Terent & Andersson) 359

Surgery

- Elevated bone phosphorus/hydroxyproline ratio following jejunoileal bypass surgery (Hey Tougaard) 371
- The diagnosis of atrial myxomas (Schlossman Selin Wallin & Wallentin) 349

Thymus

- Immunological studies on human thymus (Christensson Matell & Biberfeld) 161

Thyroid

- Serum reverse T₃ determinations in the laboratory diagnosis of hyperthyroidism (Ljunggren & Hallner) 91
- Multifocal idiopathic fibrosclerosis (Lærhammer Nielsen) 119
- Cortisone induced remission of hypothyroidism in Schmidt's syndrome (Draminsky Petersen & Bergman) 125
- High immune responsiveness in a family with multiple paraproteinaemia and autoimmune thyroid disease (Weits de Gast The van der Giessen Ockhuizen Festen & Mandema) 169
- The epidemiology of thyrotoxicosis in Denmark (Mogensen & Green) 183
- Effect of verapamil on blood glucose and serum insulin in patients with hyper and hypothyroidism (Andersson & Rydmark) 375

Treatment

- Plasmapheresis—Bloodletting revived and refined (Waldenström) 1
- The effects of cimetidine (Tagamet®) on renal function in patients with renal failure (Larsson Bodemar Kågedal & Wälan) 27
- Effect of guar gum on body weight and serum lipids in hypercholesterolemic females (Tuomilehto Voutilainen Huttunen Vuorio & Homan) 45
- Hydralazine in arterial hypertension (Wulff Lenz Krosgaard & Holst) 49
- Antiarhythmic properties of a neuroleptic butyrophonone melperone in acute myocardial infarction (Mogelang Petersen Folke & Olesen) 61
- Cortisone induced remission of hypothyroidism in Schmidt's syndrome (Draminsky Petersen & Bergman) 125
- Symptomatic diabetes mellitus cured by potassium and withdrawal of polythiazide in a hypotensive hypertensive woman (Nilsson) 129
- Effect of long term treatment with human leukocyte interferon on various laboratory parameters (Ingemarsson Bergström Broström Cantell & Strander) 155
- HDL increasing effect of cyclofenil (Persson & Fex) 209
- Urinary zinc excretion during treatment with different diuretics (Wester) 205
- Effect of metoprolol on QT_c intervals after acute myocardial infarction (Ahne Erhardt Lundman Rehnqvist & Sjogren) 23
- Excessive sensitivity to the hyponatremic effect of chlorpropamide in a patient with diabetes mellitus and anterior pituitary insufficiency (Aasen & Frey) 233

To treat or not ■■ treat—is this the question? (Waldenström)	241
Zinc balance before and during treatment with beraprost, methuazide (Wester)	265
Tissue zinc at autopsy—Relation to medication with diuretics (Wester)	269
Effects of nifedipine treatment in hypertension (Henningsen, Bergergren, Malmberg, Pål, Renmarker & Strand)	273
Psychological and social problems encountered in active treatment of chronic uraemia. III (Hirvas, Enckell, Kuhlback & Pasternack)	285
Blood glucose control and lipolysis in diabetes mellitus (Arner, Ergfeldt & Östman)	297
Comparative single-dose kinetics and effects of four sulfonylureas in healthy volunteers (Sartor, Melander, Schersten & Wåhlin-Boll)	301
Membranous glomerulopathy in a patient on captopril (Hoomijne, Dorber, Prins & Weening)	315
Granulomatous hepatitis in a patient receiving carbamazepine (Levanter)	333
The outcome of patients with transient ischaemic attacks and stroke treated with anticoagulants (Terent & Andersson)	349
Effect of verapamil on blood glucose and serum insulin in patients with hyper- and hypothyroidism (Andersson & Roydmark)	375
Renal function and morphology in long-term lithium and combined lithium-neuroleptic treatment (Bucht, Wåhlin, Wentzel & Winblad)	381
Circulating protein complexes in D-penicillamine therapy of rheumatoid arthritis (Vorberg, Woldheim & Gedda)	393
24-Hour blood glucose profiles in insulin-dependent diabetes treated with intravenous insulin infusion systems (Deckert, Boysen, Sandahl, Christiansen, Knudsen, Aaby, Svendsen & Andersen)	451
Emergency treatment of severe hypertension evaluated in a randomized study (Danish Multicenter Study)	473
Amiloride induced hyponatremia (Tarssanen, Huikko & Rosu)	491

Tumours

Effect of long term treatment with human leukocyte interferon on various laboratory parameters (Ingmarsson, Bergström, Broström, Cantell & Strandberg)	155
The diagnosis of atrial myxomas (Schlossman, Selin, Wallin & Wallentin)	309

Uraemia

Psychological and social problems encountered in active treatment of chronic uraemia. III (Hirvas, Enckell, Kuhlback & Pasternack)	285
--	-----

Urine

Demonstration and partial characterization of an atypical protein in the urine of a patient with primary amyloidosis (Wille, Olsen, Førre, Sletten & Jentoft)	177
Increased urinary protein excretion after intravenous injection of furosemide in man (Vitala, Pasternack, Kallio, Vantinen & Metsä-Ketelä)	279
Urinary excretion of inorganic sulfate, ester sulfate, total sulfur and taurine in cancer patients (Baldertorp & Mårtensson)	293
Haematuria in a population sample of women (Bengtsson, Bengtsson & Lennquist)	417

Veins

Aneurysm of the popliteal vein as a cause of pulmonary embolism (Persson, Eklof & Wintzell)	
---	--

Vitamins

Vitamin D deficiency in welfare institutions for the aged (Toss Almquist Larsson & Zetterqvist)	87
Castle's test (with vitamin B ₁₂ and normal gastric juice) in the ileum in patients with genuine and patients with tapeworm pernicious anaemia (von Bonsdorff & Gordin)	193

Zinc

Urinary zinc excretion during treatment with different diuretics (Wester)	709
Zinc balance before and during treatment with bendroflumethiazide (Wester)	265
Tissue zinc at autopsy—Relation to medication with diuretics (Wester)	269

LIST OF AUTHORS

Åberg H 467	von Bonsdorff B 193	Fex G 205
Aaby Svendsen P 451	Born G V R Suppl 642	Førre Ø 177
Aagenæs Ø 389	Bowie E J Suppl 642	Forster W Suppl 642
Åkesson B 367	Brandt L 13	Fosså S D 137
Aasen G 233	Britton M 81	Folke P E 61
Abdon N J 69 73	Broström L Å 155	Fowler S Suppl 642
Abdulla M 367	Bucht G 381 387	Frey H M M 233
Adamson U 101		Frøyshov Larsen I 111
Adolfsson R 387	Camejo G Suppl 642	Furberg B 77
Afzelius B A 145	Cantell K 155	Fuster V Suppl 642
Agradi E Suppl 642	Carlens P 219	
Ahnve S 55 223	Carvalho A C A Suppl 642	Gallit C Suppl 642
Antonietti S 495	Chignard M Suppl 642	de Gast G C 169
Almqvist S 87	Christensson B 161	Gedda P O 393
Andersen A R 451	Christiansen C 229	van der Giessen M 169
Andersen E Suppl 643	Cortez M M Suppl 642	Gordin R 193
Andersson B 349	Le Couedic J P Suppl 642	Granberg P O 101
Andersson D E H 375		Grande P 229
Andersson R 77	Dahlström U 425	Green A 183 401
Andren L 481	Danish Multicenter Study 473	Grimelius L 95
von Arbin M 81	Davies J A Suppl 642	Guldbrandsson T 481
Arner P 297	Davies M J Suppl 642	Gutniak E 95
Asplund J 331	Deckert T 451	Gutniak M 95
Aurell M 17	Dencker I 367	
	Donker A J M 325	Hallgren R 33
Baldetorp L 293	Donner M 407	Halonen P I 41 Suppl 642
Bengtsson C 417	Draminsky Petersen H 125	Hamilton D V 337
engtsson U 417		Hanssen J F 369
Benveniste J Suppl 642	Efendić S 101	Hansson L 481
Bergengren B 273	Ejvinsson G 445	Hartford M 17
Berglund G 17	Eklof B 407	Hedstrand H 467
Bergman M 125	Enckell M 285	Hellerstedt M 213
Bergström K 155	Engfeldt P 297	Helmers C 55 81
Biberfeld P 161	Erhardt L 223	Henningsen N C 273
Björkerud S Suppl 642	Erkssen J 199	Hey H 321
Bjornstad Pettersen H 397	Erksson I 345	Himberg J J Suppl 642
Blohmé G 309	Ewetz L 145	Hirvas J 485
Blomhoff J P 199		Hokfelt T 95
Bodemar G 27	Fahrenkrug J 95	Holm G 187
Boysen J 451	de Faire U 81	Holmgren A 219
Bone D 219	Festen J J M 169	Holst B 49

- Homan K 45
 Hoornijse S J 325
 Huikko M 491
 Huttunen J 45

 Ikkala E Suppl 642
 Ingmarsson S 155

 Jagerblad M 367
 Jentoft H 177
 Johansson S 187
 Johnsson H 219
 Jonasson R. 213 219
 Jones S P 337
 Julstrud O J 137

 Kagedal B 27
 Kallho S 279
 Kallner G 91
 Kandilas J 219
 Karlsson K 309
 Kaukola S 41
 Kaulough-Rathbone R L. Suppl 642
 Kølendorf K 451
 Kosunen T U 495
 Kræmmer Nielsen H 119
 Krogsgaard A R. 49
 Kronmann V 401
 Kuhlback B 285

 Lampe S 5
 Larsson L 87
 Larsson R. 27
 Lauvuoja K Suppl 642
 Lea F J A 337
 Lees R S Suppl 642
 Lenz K 49
 Levander H G 333
 Lidbeck J 459
 Lincoln K 417
 Lindström E 289
 Lindström F D 289 425
 Lindvall N 101
 Lins P E 101
 Lithell H 467
 Lithner F 315 387
 Ljunggren J G 91
 Ljungman S 17
 Low H 101
 Lopez F Suppl 642
 Lundberg J M 95
 Lundin L 33
 Lundman T 55 223
 Lundqvist G 95
 Luomanmaki K 133

 Luurila O J Suppl 641
 Ly B 111 115
 Lyager-Nielsen B 343 463

 Martensson J 293 425
 Mackay I R 411
 Malmberg O 273
 Mandema E 169
 Manninen V 41 Suppl 642
 Markosyan R A Suppl 642
 Matell G 161
 Melander A 301 367
 Metsä-Ketela T 279 Suppl 642
 Miah K 81
 Miettinen M 431
 Mogelvang J C 61
 Möller M 65
 Mogensen E F 183
 Mogensen L. 219
 Moncada, S Suppl 642
 Mosquera B Suppl 642
 Murray V 81
 Murros J 133
 Mustard J F Suppl 642

 Nilsson B E 69 73
 Nilsson G 129
 Nilsson I M 5
 Nilsson P G 13
 Norberg R 393
 Norden Å 367
 Nordlander R 219
 Nordøy A Suppl 642

 Ockhuizen T 169
 Östman J 297
 Olofsson B O 77
 Olsen E 177
 Orinius E 213 219 445
 Ove en L 61

 Paasonen M K Suppl 642
 Pahlham M A Suppl 642
 Pahlm O 355
 Palmblad J 145
 Pape E 137
 Pasternack A 279 285
 Paulley J W 257
 Penttilä O Suppl 642
 Persson B 205 237
 Persson B G 407
 Peters M 411
 Petersen E N 61
 Petersson B 407
 Petroni A Suppl 642
 Pettersson T 495

 Pihl O 273
 Pitkanen T 495
 Ponka A 495
 Prins E J L 325

 Rast V Suppl 642
 Rausing A 237
 Rehnqvist N 223
 Reitan J B 137
 Renmarker K 273
 Rojdmärk S 375
 Roketus A 95
 Rømer F K 437
 Ronnemaa T Suppl 642
 Rorvik T O 111 115
 Rosell S 95
 Rosenqvist U 95
 Ross R. Suppl 642
 Rossi M 491
 Roxin L E 33

 Sandahl-Christiansen J 491
 Sartor G 301
 Sauar J 199
 Saxen E 431
 Saxen L. 431
 Schersten B 301 367
 Schlossman D 349
 Schöhr P 229
 Schroll M 245
 Selin K 349
 Simonsen E. 343 463
 Sivertsson R 481
 Sjögren A 223
 Skrede S 199
 Sletten K 177
 Slettnes O N 137
 Smith U 187
 von Smitten K Suppl 642
 Socini A Suppl 642
 Solorro L Suppl 642
 Solatunturi E Suppl 642
 Solheim D P 137
 Starova R Suppl 642
 Stavem P 111 115
 Stenman S Suppl 642
 Stjernberg N 397
 Stormorken H Suppl 642
 Stræde Nielsen J 343 463
 Strand L 273
 Strander H 155
 Sundblad R 95
 Svensson A 481

 Tarssanen L. 491
 Terent A 359

- | | | |
|-----------------------|-------------------------|-------------------|
| Thayssen P 65 | Vargaftig B B Suppl 642 | Wikstrand J 17 |
| The T H 169 | Vedin A 187 | Wilhelmsen L 17 |
| Thompson G 341 | Venge P 33 | Wilhelmsson C 187 |
| Thulin T 367 | Venzelos N 145 | Wille L 177 |
| Tornblom N 315 | Vinni S 45 | Winblad B 381 387 |
| Torstula I Suppl 642 | Voutilainen E 45 | Wintzell K 407 |
| Toss G 87 | | Wollheim F A 393 |
| Tougaard L 321 | Wählin Boll E 301 | Wood C Suppl 642 |
| Truedsson H 397 | Wahlm A 381 | Woolf N Suppl 642 |
| Tuomilehto J 45 | Walan A 27 | Wulff K 49 |
| Turesson I 237 | Waldenstrom J 309 | |
| | Waldenstrom I G I 241 | Ylitalo P 279 |
| Uden A M 145 | Wallentin I 349 | |
| | Wallin J 349 | Zetterqvist H 87 |
| Vaaler S 389 | Weening J J 325 | Zettervall O 237 |
| Vanttinen T 279 | Wehmeyer A Suppl 642 | |
| Vaheri A Suppl 642 | Weiss J 169 | Å see Aa |
| Vane J R Suppl 642 | Wentzel T 381 | A see Ae |
| Vapaatalo H Suppl 642 | Wester P O 209 265 269 | Ö see Oe |
| | | Ø see Oe |

